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CANCER RESEARCH

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CANCER RESEARCH
A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 8 MARCH, 1948 NUMBER 3

Attempts to Produce Cancer in Rhesus Monkeys with Carcinogenic Hydrocarbons and Estrogens*

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(Received for publication August 20, 1947)

By 1936 a great amount of work had been done on cancer in the lower mammals, the results of which were being used with increasing frequency in attempts to interpret human cancer, yet very little was known about cancer in other primates. The only experimental study in this regard was that of Bonne, Lodder and Streef (2) on the effects of cutaneous application of coal tar on the Javanese monkey. It therefore seemed that a careful investigation should be made of the effects of carcinogenic hydrocarbons and estrogens on the Rhesus monkey. Despite the large numbers of Rhesus monkeys that have been under observation for long periods of time, including the latter half of the life span, only 2 cases of spontaneous cancer have been described in this monkey, an osteogenic fibroma (1) and a prostatic carcinoma (6). This paucity of reported cases would suggest a low susceptibility to cancer but cannot in itself be considered adequate proof that such is true. In the study to be reported here it soon became evident that cancer would not be induced by brief periods of treatment. Therefore, a relatively large number of monkeys was set up for chronic treatment to continue until the animals died. No cancers have been observed, and the present time was arbitrarily chosen to report the results of these experiments, since only 4 animals remain alive. Of these 4, 3 have been treated for 8 to 10 years and are still in good health.

MATERIAL AND METHODS

For this experiment, which has run from 1936 to the present time, 50 Rhesus monkeys were used. Three of these monkeys were mature males; the rest were females. Twenty-one of the females were castrated and 3 were hysterectomized. They were purchased from a commercial dealer. It is estimated that the monkeys were between 3 and 10 years of age when treatment was begun, although some may have been older. They usually weighed between 4 and 5 kilos. During the winter the animals were kept indoors. No attempt was made to keep the room at a constant temperature, the temperature probably fluctuated from 70° to 85° F. In the summer the monkeys were kept in outside enclosed paddocks open to the sun and air.

The type of food varied a great deal during the period of treatment but was controlled to the extent that the animals remained in good health and maintained or gained weight depending on the age of the individual animal. From 1936 to 1938 the diet was made up of a variety of fruits and vegetables, but thereafter a more standardized diet was used. This consisted of prepared dog meal and pellets which provided an adequate supply of all of the known vitamins except vitamin C. The latter was supplied by feeding canned tomatoes, oranges or fresh cabbage. In 1938 Eastern States Dog Food was fed during the winter as a mash supplemented with canned tomatoes. During the summer months this food was given in pellet form, supplemented with oranges. During 1939 Purina Fox Chow and later Purina Dog Chow were substituted for the Eastern States Dog Food, primarily because of taste preference. Since 1942 all of the prepared dog food has been fed in pellet form and vitamin C has been supplied almost exclusively by feeding fresh cabbage. During the entire period the monkeys were fed Chim Crackers (Kennel Food Supply Co.) once or twice a week for variety. Water was available at all times.

Nearly all the organs and tissues that frequently become cancerous in the human being were treated with carcinogenic hydrocarbons and estrogens. The specific treatment for each animal is given in Table I. Both systemic and local applications were used.

* This investigation was supported by grants from the International Cancer Research Foundation.

† Deceased February 3, 1943. This investigation was started under the direction of Professor Allen. At the beginning the senior author served only as an assistant, but later he was given the responsibility for the execution of the program. Since Professor Allen's death in 1943 the investigation has been continued by the senior author, who has also compiled the data and prepared the manuscript.

Estrogens and carcinogens were administered directly to the breasts. In several animals a specific carcinogen was injected directly into one or more of the 8 or 10 ducts opening into the nipple so that a particular sector was impregnated with the carcinogen.

Estrogens.[1]—A variety of estrogens was used. At the beginning of the experiment estradiol benzoate (10,000 I.U. per cc.) and theelin (1,000 I.U. per cc.) in sesame oil were employed. In 1938 estradiol dipropionate (50,000 I.U. per cc.) was substituted for the theelin and estradiol benzoate. In the same year, when synthetic estrogenic substances became available, one animal was started on triphenylethylene and one on diethylstilbestrol. At this time all estrogens were dissolved in oil and injected subcutaneously or intramuscularly into the region of the thigh except where injections were made directly into the breast. In general 5,000 I.U. were injected twice weekly but a considerable range of dosage was represented in the animals treated (Table I). Beginning in 1938 estradiol dipropionate in pellets was administered to one animal. Most of the estrogen-treated animals started after 1939 received either estradiol dipropionate or estrone in pellets placed subcutaneously in the breast and thorax. After 1942 all monkeys that were still on chronic estrogen treatment were implanted with pellets of 30 per cent stilbestrol in cholesterol and reimplanted as soon as the estrogen effects began to fade.

Carcinogenic hydrocarbons.[2]—The carcinogens used were methycholanthrene, benzpyrene and dibenzanthracene in crystalline form. For subcutaneous injection they were dissolved in sesame oil in such concentration that each cc. contained 10 mgm. of carcinogen. It was necessary to keep the dibenzanthracene in an incubator in order to keep it in solution. These three substances were also painted on the skin from a benzene solution (10 mgm. per cc.). In addition dibenzanthracene was painted from a methyl salicylate solution (10 mgm. per cc.). Injections of one mgm. of carcinogen in oil were usually made twice weekly. Cutaneous applications were made once or twice a week, but this method of application, together with the volatile nature of the solvent, made it difficult to determine the exact amount of carcinogen administered. It was estimated, however, to be about 1 mgm. per application. Usually more than one site of injection as well as painting was used at the same time, so the weekly dose of carcinogenic hydrocarbons varied from 2 to 6 mgm. Other methods of application were also used. Methycholanthrene was implanted in paraffin pellets or in capillary tubes. Also crystals of methylcholanthrene were dusted into the peritoneal cavity, sewed into an omental pocket or placed over the uterus and held in place by suturing the omentum to the uterus. Pellets of methylcholanthrene and benzpyrene were placed subcutaneously, into the myometrium, into the cervix, into the submucosa of the vagina, under the serosa of the stomach, or into the mammary glands. These were introduced by means of a number 13 gauge trocar. Methylcholanthrene was suspended in horse serum for intravenous injection and was given orally in large doses to one animal. The oral application was made either by stomach tube or fed in glucose solution. Beginning in 1943 the respective carcinogens were given in pellet form to all of the chronically treated animals because the areas where injections and paintings had been made were so scarred that it was doubtful that much of the carcinogen was retained following injection, or absorbed following painting.

OBSERVATIONS

Since the animals received such varied treatment and each treated area gave a variety of responses, abbreviated protocols will be given for 2 monkeys which have been used rather extensively as material for illustrating this paper. These protocols will be given before the general description, since for brevity it is necessary to condense the observations under the headings of the various chemicals used.

Abbreviated Protocol I

Animal: #66. *Age:* mature. *Sex:* ♀. *Condition:* excellent. *Weight:* 4,600 gm. *Date started:* Jan. 1, 1937. *Previous operations or treatment:* none. *Carcinogenic treatment:* see Table II.

Lesions: Breasts.—Within 2 months both nipples had doubled in size and were firm. A ligneous induration extended out radially from each nipple and the skin became firmly affixed to the underlying tissue. After 7 months small abscesses formed and ulcerated, first in the right breast and then in both breasts. This effect became so extreme as to necessitate intermittent treatment during the following months. When healing, the ulcerated areas

(Continued on Page 100)

[1] The theelin and estrone were kindly supplied by the Parke Davis Co. through the courtesy of Dr. Oliver Kamm. The estradiol benzoate was supplied by the Schering Corporation through the courtesy of Dr. Erwin Schwenk. The estradiol dipropionate was supplied by Ciba Pharmaceutical Co. through the courtesy of Mr. R. C. Mautner. Triphenylethelene and diethystilbestrol were supplied by E. R. Squibb & Son through the Courtesy of Dr. J. A. Morrell.

[2] The methylcholanthrene and 1,2,5,6 dibenzanthracene were purchased from Eastman Kodak Co., Rochester, New York, and were used as prepared in their research laboratories. It is possible that early in the experiment 9,10-dimethyl-1,2-benzanthrene was used instead of the 1,2,5,6-dibenzanthracene. The benzpyrene was purchased from Hoffman-LaRoche and Co. This chemical was prepared in Basel, Switzerland.

TABLE I: METHOD AND PERIOD OF TREATMENT OF MONKEYS WITH ESTROGENS AND CARCINOGENIC HYDROCARBONS

Number and sex		Methylcholanthrene	Dosage* Weekly mgm.	Dosage* Total gm.	Benzpyrene	Dosage* Weekly mgm.	Dosage* Total gm.	Dibenzanthracene	Dosage* Weekly mgm.	Dosage* Total gm.	Estrogen	Dosage* Weekly mgm.	Dosage* Total gm.	Period of Treatment
1	♀	.....			.....			Rt. breast,[1] abdomen[1]	6.0	0.150	.....			5½ mos.
2	♀§	.....			Mammary duct,[2] lt. nipple,[2] uterus[2]		0.073	.....			Thigh intramuscularly[2]	0.07	0.55	2 mos.
3	♂	Rt. nipple,[4] abdomen,[2] lt. ear[2]		0.032	Abdomen[2]		0.012	Abdomen,[2] rt. ear[2]		0.030	.....			7 wks.
4	♂	Lt. ear,[2] lt. inguinum[3]	1.0	0.351	Rt. ear,[5,2] rt. breast,[5] rt. abdomen,[5] rt. scrotum[5]	2.0	0.117	Lt. ear,[5] lt. breast,[5] lt. abdomen,[5] lt. scrotum[5]		0.090	.....			1 yr. 5½ mos.
5	♂	Base of tail[2]	1.0	0.107	.....			.....			Thigh intramuscularly[2]	1.00	107.00	2 yrs. 1 mo.
6	♀§	.....			.....			.....			Subcutaneously[2]	0.40	10.88	6 mos.
7	♀§	.....			.....			.....			Subcutaneously[2]	0.24	14.68	2 yrs.
8	♀	.....			Vagina,[2] breast,[2] buttock,[2] rt. ear,[5] lt. ear[2]	4.0	0.464	.....			Thigh intramuscularly[2]	1.00	110.00	2 yrs. 9 mos.
10	♀	.....			.....			Lt. breast[2]	6.0	0.133	.....			12½ mos.
12	♀	.....			Uterus[2]		0.007	.....			Thigh intramuscularly[2]	1.00	7.05	58 days
12a	♀§	.....			Uterus,[5] vagina,[5] breast,[5] sex skin,[5]		0.300	.....			.....			10 wks.
13	♀§	.....			Lt. breast,[2] uterus[2]		0.032	.....			Subcutaneously[2]		0.10	4 wks.
14	♀§	.....			Rt. breast,[2] vagina[2]		0.112	.....			Subcutaneously[2]		0.20	4 wks.
15	♀	.....			.....			.....			Subcutaneously[2]	0.24	7.28	8 mos.
16	♀	.....			Rt. breast[2]		0.028	.....			Subcutaneously[2]		0.085	5 mos.
19	♀§	Base of tail,[2] margin sex skin[2]	2.0	0.300	.....			.....			Subcutaneously[2]	1.00	188.00	4 yrs.
20	♀§	.....			Lt. breast,[2] rt. hip[2]	6.0	0.140	.....			Intramuscularly[2]	0.70	16.05	5 mos.
28	♀	Omentum,[6] under rectus sheath[6]		0.050	.....			.....			.....			9 mos.
33	♀§	.....			Lt. breast,[2] uterus[2]		0.261	.....			.....			8 mos.
41	♀§	.....			Perivaginal,[2] lt. breast[2]	8.0	0.096	.....			Subcutaneously[2]	0.20	2.20	2 mos.
42	♀§	.....			.....			Lt. breast,[2] rt. buttock[2]	5.0	0.452	Intramuscularly[2]	0.20	12.60	2 yrs.
43	♀§	Lt. breast,[10,2] skin[7]	2.0	0.084	.....			.....			.....			10½ mos.
51	♀§	.....			Uterus,[2] rt. tube,[2] lt. breast,[2] thigh[2]	10.0	0.122	.....			Subcutaneously[2]	0.60	8.00	3 mos.
52	♀	.....			.....			.....			Subcutaneously[2]	0.80	8.55	3 mos.
53	♀	.....			.....			.....			Subcutaneously[2]		0.13	7 mos.
54	♀§	.....			.....			.....			Subcutaneously[2]	1.00	8.55	2 mos.
59	♀	.....			.....			.....			Subcutaneously[2]	0.16	8.83	1 yr. 3 mos.
61	♀	.....			Uterus,[2] rt. tube,[2] lt. breast,[2] thigh[2]	2.0–4.0	0.350	.....			.....			4 yrs. 3 mos.
63	♀	Rt. breast[2]	2.0	0.025	.....			Lt. breast,[2] lt. ear,[2] rt. ear[2]	5.0	0.225	.....			11 mos.
66	♀	Interscapular area[7]	2.0	0.380	Rt. ear,[2] rt. breast[2]	2.0	0.430	Lt. ear,[2] lt. breast,[2] back,[7] perianal[7]	5.0	5.323	.....			4 yrs. 10 mos.
90	♀	.....			.....			.....			Subcutaneously[2]	0.16	11.94	1 yr. 8 mos.
92	♀	Uterus,[5] skin,[5] lt. breast,[2,5] lt. ear,[2] rt. ear,[7,2]		1.615	Rt. ear,[2,2] rt. breast,[2,2] abdomen[2,2]	4.0	0.700	.....			.....			10 yrs. 6 mos.
96	♀§	Lt. ear,[7] tail,[7] cervical canal[9]	6.0	0.820	.....			.....			Intramuscularly[2]		2.60	3 yrs. 3 mos.
104	♀	Lt. breast,[2] abdomen[2]	5.0	0.042	Abdomen[2]	5.0	0.042	Rt. breast,[2] lt. breast[2]	4.0	0.028	.....			9 wks.
106	♀§	Intravenously[11]	5.0	0.025	.....			.....			Intramuscularly[2]	0.60	48.00	1 yr. 6 mos.
107	♀§	Omentum[6]		0.055	.....			.....			Intramuscularly,[2] uterus,[2] abdomen[2]		150.00	3 yrs.
146	♀	Lt. thigh[2,7]		0.096	.....			.....			.....			6 mos.
149	♀§	Cervix,[2] breasts[2]		0.100	.....			.....			.....			1 yr. 9 mos.
151	♀§	.....			.....			.....			Thorax,[2,2] abdomen[2,2]		298.00	3 yrs. 1 mo.
173	♀§	Cervical stump[2]		0.100	.....			.....			Thigh,[2] thorax[2]		120.00	2 yrs. 9 mos.
174	♀	.....			.....			.....			Thigh intramuscularly[2]		30.00†	2 yrs. 8 mos.
175	♀	.....			.....			.....			Intramuscularly[2]	6.00	155.00	6 mos.
177	♀	.....			.....			.....			Intramuscularly[2]	1.00	109.00‡	2 yrs.
179	♀	.....			.....			.....			Intramuscularly[2]	1.00	121.00	2 yrs. 4 mos.
180	♀	Uterus,[2] cervix[2]		0.135	.....			.....			Thigh,[2] uterus,[2] omentum[6]		127.00	5 yrs. 1 mo.
189	♀	.....			.....			.....			Thorax,[2,2] thigh[2,2]	3.00	591.00	5 yrs.
274	♀	Rt. breast,[10,2] lt. breast,[2,7] ears,[2,7] abdomen,[2] thorax,[2] femur[2]	2.0–4.0	1.055	.....			Lower lip[2]		0.072	.....			10 yrs. 9 mos.
705	♀§	Lt. breast,[2,7,2] lt. ear,[2,7,2]		1.030	Rt. ear,[2,7,2] rt. breast,[2,7,2]		0.510	.....			.....			9 yrs.
942	♀	Cervix,[2,9]	1.0	0.072	.....			.....			.....			2 yrs. 6 mos.
984	♀	Orally		1.000	Serosa of stomach[2]		0.080	.....			.....			3 yrs.

* The weekly dose is difficult to estimate in all cases as it was often varied. Therefore, the weekly dose most frequently used is given in the table. Sometimes the injections were omitted for short periods to allow animals to regain health. If the weekly dose varied too much, no figure is listed. It is for these reasons that the total and weekly doses frequently do not agree. The total dose is the accurate figure.

The methods of administration of the chemicals are keyed as follows: [1] injected in saline suspension, [2] injected in sesame oil, [3] pellets, [4] crystals in a capillary tube, [5] injected in melted paraffin or implanted as paraffin pellets, [6] crystals inserted into organs surgically, [7] painted in benzene, [8] painted in oil, [9] instilled into the cervical canal, [10] injected directly into the lactiferous ducts, and [11] injected in horse serum.

† 5.8 gm. of triphenylethelene were also given.

‡ Also received 75 mgm. of testosterone propionate.

§ Castrated.

showed a tendency for keloid formation. Irregular pigmentation and fibrotic reactions persisted after healing.

During the second year there was little change in the breasts; both continued to show marked induration surrounding the nipples. During the third year the dibenzanthracene treatment in the left breast maintained it in a chronic state of inflammation at the injection site. The left breast continued to show much greater reaction to the carcinogen than did the right breast which received benzpyrene. However, a mass having the characteristics of a fibroma developed in the right breast. It was apparently non-tender, and was fixed to the overlying epidermis. During the fourth year this growth resolved itself into two distinct masses, one below the nipple and fixed to the overlying epithelium and the other in the superior lateral quadrant and fixed to the underlying tissue. Due to the marked induration and scar formation of the left breast, the left nipple became twice as large as that of the right and was slightly cyanotic and colder. These conditions (Fig. 1) were maintained until the animal came to autopsy.

Left ear.—Injections into the left ear were begun in January, 1937; the helix and antitragus of the pinna were the sites of injection. Fibrous tissue masses were induced rather rapidly, and ulcerations not infrequently produced. In August, 1939, the injection site was changed to the anterior portion of the pinna adjacent to the point of attachment to the head, and to the area just anterior to the attachment of the pinna. This area was anatomically able to tolerate the injections of the foreign material. A mass developed here and gradually increased in size until February, 1940, when it underwent internal necrosis with the discharge of thick grumous material from several sinuses. The injection site was then moved slightly anterior to the tragus, where a cleanly circumscribed mass developed which was in part subcutaneous fibrous proliferation and in part epithelial proliferation. The surface of this mass desquamated, and, if rubbed, left small bleeding points beneath the easily detachable cornified layer. The old injection sites remained readily visible as scarred desquamated or proliferated areas (Fig. 4). This condition remained until autopsy.

Right ear.—The right ear was also started on treatment in January, 1937, but with benzpyrene, the injection sites being the same as those for the left ear. There was little reaction during the early treatment, but pressure necrosis resulting from the instillation of the oily material into this site occurred at intervals. In August, 1939, when the injection site of the left ear was shifted, the injection site in the right ear was moved to the anterior portion of the helix adjacent to the point of attachment, and to the skin at, and anterior to, the tragus. A large, firm mass resulted at this point (Fig. 5). A well demarcated epithelial reaction over this mass was also observed. The skin thickened and desquamated but did not ulcerate. The reaction of the skin, though definite, was not as intense as that observed with other carcinogens. The fibrous tissue mass beneath the area of skin reaction was, however, quite marked and exhibited no tendency toward necrosis. These conditions persisted without any further reaction until autopsy.

Interscapular and perianal regions.—The reaction to painting was slower in appearing than the reaction following injections. However, marked hyperkeratinization, desquamation and small nodule formation was present by 6 months. This persisted throughout the period of treatment, increasing gradually in degree so that, passing through a state of formation of small keratoses and small areas of fine punctate cornification, the area ultimately became covered with a thick, tough layer composed of dried carcinogen and desquamated epithelium. Within 1½ to 2 years this layer became densely adherent to the living skin, and its removal caused small bleeding points. In June, 1938, painting with methylcholanthrene in benzene was substituted for dibenzanthracene in benzene on the interscapular area, and methylsalicylate was substituted for benzene as a solvent for dibenzanthracene for painting the perianal region. During the early treatment there was no appreciable loss of hair, but gradually the hyperkeratinization of the hair follicles and sebaceous glands became quite extreme. Localized areas of extreme epidermal proliferation occurred late in the treatment and persisted until autopsy.

TABLE II: CARCINOGENIC TREATMENT OF MONKEY NO. 66

Carcinogens*	How applied	Date started	Areas treated	Amount weekly mgm.	Total gm.
DBA	injected in oil	1/1/37	left ear	1	0.323
DBA	" " "	"	left breast	1	
BP	" " "	"	right ear	1	0.430
BP	" " "	"	right breast	1	
DBA	painted in benzene	"	interscapular	10	3.180
DBA	" " "	"	perianal	10	
MC	" " "	6/1/38	interscapular	2	0.380
DBA	painted in methylsalicylate	"	perianal	10	1.820

Total: Mc, 0.380 gm.; BP, 0.430 gm.; DBA, 5.323 gm.; Estrogen, none.

* DBA, dibenzanthracene; BP, Benzpyrene; MC, Methylcholanthrene.

Weight changes: May 20, 1938, 5,127 gm.; March 18, 1939, 5,660 gm.; Jan. 2, 1940, 5,580 gm.; March 8, 1941, 5,665 gm.

Photographs: All treated areas were photographed June 15, 1940.

Cause of death: Paratyphoid. Died Oct. 11, 1941.

Autopsy: Gross findings. After July, 1940, all injections were made in such a way as not to disturb the areas of reactions any more than necessary, with the result that at autopsy there were no ulcerated areas of necrosis. The hyperkeratinization and fibrotic masses described above were still present, although the skin appeared more normal in texture and the fibrotic masses had a more flexible feel. The animal had a heavy layer of subcutaneous fat and showed no evidence of emaciation. The digestive tract was typical of all other animals dying of dysentery. All other organ systems were negative as to pathology. The ovaries and uterus were quite small for an adult female. The cervix appeared to be greatly fibrosed.

Histological findings.—The skin in the treated areas was about 4 times as thick as normal skin. In addition to a piling up of the epithelium, there were nests of squamous cells with occasional pearl formation. This proliferation was orderly, however, and the basement membrane was intact throughout. The epithelium contained occasional mitotic figures. The fibrotic masses consisted of moderate

proliferations of mature fibroblastic elements and a minimal infiltration of lymphocytes and plasma cells. The entire treated area showed a diffuse pattern of increased numbers of collagenous fibers with dispersed small areas of chronic inflammation. The older treated areas showed typical scar tissue. The painted areas showed the typical extreme hyperkeratinization of the surface epithelium, hair follicles and sebaceous glands (as illustrated in Fig. 19), and there was a diffuse increase in the connective tissue of the subdermal tissues, producing a dense, non-elastic condition.

The connective tissue in both breasts was dense and non-elastic (Fig. 14). The nipples were quite fibrotic, the left one showing much more edema than the right one. The terminal ducts of the mammary glands were normal, and the alveolar system was appreciably stimulated, especially in the right gland (Fig. 16). The lactiferous ducts of the right mammary gland showed extreme metaplasia (Fig. 15). The ovaries were atrophic, the cervix was fibrosed, but the glands of the uterus were moderately well developed, despite the marked atrophy of the ovary.

TABLE III: CARCINOGENIC TREATMENT OF MONKEY NO. 705

Carcinogens*	How applied	Date started	Areas treated	Amount weekly mgm.	Total mgm.
MC	injected in oil	6/14/38	left breast	1	182
MC	" " "	6/10/38	left ear	1	182
MC	painted in benzene	"	left breast	2	364
MC	" " "	"	left ear	2	364
BP	injected in oil	"	right ear	1	182
BP	" " "	"	right breast	1	182
MC	pellets	12/2/41	left breast		25
MC	"	"	base of left ear		25
BP	"	"	right breast		25
BP	"	"	right ear		25
MC	"	11/43	left breast		25
MC	"	"	left ear		25
BP	"	"	right breast		25
BP	"	"	right ear		25
MC	"	3/16/45	left breast		25
MC	"	"	left ear		25
BP	"	"	right breast		25
BP	"	"	right ear		25
MC	"	5/5/47	left breast		25
MC	"	"	left ear		25
BP	"	"	right breast		25
BP	"	"	right ear		25

Total: MC, 1.292 gm.; BP, 0.564 gm.; DBA, none; Estrogen, none.

*MC, methylcholanthrene; BP, benzpyrene; DBA, dibenzanthracene.

ABBREVIATED PROTOCOL 2

Animal: #705. *Age:* mature. *Sex:* ♀. *Condition:* excellent. *Weight:* 4,500 gm. *Date started:* June 10, 1938. *Previous operations or treatment:* ovariectomized March 1938. *Carcinogenic treatment:* see Table III.

Lesions: Breasts.—Reaction to the injected carcinogens developed gradually. At 4 months both breasts showed the formation of non-inflammatory fibrous masses at the site of injection, and, in contrast to the usual effect, the early response in the right breast to injected benzpyrene was of greater magnitude than that of the left breast receiving methylcholanthrene. By November, 1938, some of the fibrous tissue in the right breast had resolved into a small nodule which was firm and movable. It was thought possible that it might be composed of mammary tissue. The left breast had now begun to show more striking effects. There was a thick, horizontal band, presumably derived from the epidermis, across the mammary region at the level of the nipple. None of the tissue changes involved visible inflammatory reactions. From this period on, the reaction of the left breast to methylcholanthrene increased, while that of the right breast (benzpyrene treated) remained relatively unchanged for long periods or showed periods of slow regression followed by periods of slow progression. The tissue mass in the left breast became organized into a distinct nodule. It continued to grow and presumably represented a moderate proliferation of the basal layers of the skin with infiltration of dense connective tissue. By February, 1940, a firm irregular mass had formed around the right nipple. This was firmly affixed to the overlying skin. The reaction in the left breast continued to be quite marked. There was considerable thickening of the epithelium, desquamation and loss of hair, with a vertical extension of this reaction near the nipple. By May, 1940, this surface reaction had become more widespread, and the entire painting site about the left nipple presented a picture of multiple punctate areas of proliferation of the surface epithelium with a scaling of these irregular macules and papules. This condition is illustrated in the photograph (Fig. 3) which was taken in June, 1940.

The above conditions continued until the end of 1941. By this time there was considerable thickening of the skin. This hyperkeratinization plus a deposit of the painted carcinogen made it doubtful whether the new carcinogenic applications were being absorbed. It was, therefore, decided to implant pellets of the carcinogens. The hyperkeratinization then gradually cleared up, and although the papillae and nodules still persist to some extent, they have taken on much the same consistency as the normal tissues around them. A biopsy of one of the nodules in May, 1945, showed that most of the dense fibrous tissue of the nodule had been replaced by fat.

Left ear.—From June, 1938, until August, 1939, methylcholanthrene was administered to the helix and antitragus of the pinna by injection and painting. The injections

were not well tolerated here for anatomical reasons. Inflammation was evident within a few months, and ulceration soon began. Ulceration and sloughing were so frequent that the injection site was changed (August, 1939) to the anterior portion of the helix and adjacent epithelium. Although better able, anatomically, to tolerate the injections, the new site showed pronounced reactions by October, 1939. Firm non-inflammatory fibrotic masses formed, accompanied by thickening, pitting and scaling of the epithelium. By June, 1940, this condition had progressed to the state seen in Fig. 7. The general fibrosis continued to the extent that it became almost impossible to have the injected carcinogen retained. Therefore, treatment by implantation of pellets was begun in December, 1941.

Right ear.—Benzpyrene injections into the helix and antitragus of the pinna were begun in June, 1938. The injections were poorly tolerated, but otherwise the reactions were not very marked. In August, 1938, the injection site was changed to the anterior portion of the pinna and adjacent pre-pinnal epithelium. A fibrous tissue mass formed within a month with an epithelial reaction similar to that for the right ear. By June, 1940, this had progressed to the condition seen in Fig. 6. In this ear, also, pellets replaced the injection treatment in December, 1941.

At the present time, despite the number of pellets that have been implanted, the surface reaction of both ears has completely cleared and the fibrous masses and nodules have regained almost the same consistency as the surrounding tissue.

This animal is still alive, in good health, and is being continued on treatment with implanted pellets of carcinogens.

Estrogens.—At the start of treatment there was a marked increase in the thickness of the sex skin, the edema in many cases extending over the back of the hind legs, dorsally over the crest of the ilium and forward over the forehead and face. The sex skin areas and the forehead showed a marked increase in color which usually reached a maximum within a month. After a few months of treatment both the swelling and color decreased rather slowly to a state about midway between the maximal and minimal conditions and remained at about this stage as long as estrogen was given.

Long-continued estrogen stimulation at or near the toxic level caused a loss of the normal glossiness of the hair, alopecia, loss of muscle substance, and partial atrophy of the hamstring muscles with resulting contracture of the lower limb at the thigh. The marked emaciation was followed by a cachectic condition which would result in death if sufficient rest periods were not allowed. However, there was, as could be expected, considerable variation in individual tolerance to estrogens, some animals being able to tolerate one or more milligrams weekly without the toxic effects becoming severe enough to force discontinuation of treatment.

With continued administration of smaller doses of estrogen, as has already been shown (14), menstruation occurred with quite definite periodicity even when the monkey was castrated. With reasonably large doses, the endometrium became refractory after a time and estrogen-withdrawal menstruation did not occur. One animal that received small doses of estrogen (0.05 mgm. weekly) for 3½ years, menstruated 18 days after estrogen withdrawal instead of the usual 7 to 9 days. Other animals treated for 6 months with 1.5 to 6.0 mgm. of estradiol dipropionate weekly had not menstruated by a month after estrogen withdrawal, at which time estrogen treatment was resumed. This endometrial exhaustion followed all types of estrogen treatment if the dose was high enough and the period of treatment sufficiently long.

Blood studies were made on several animals receiving fairly large doses of estrogen, but alteration of the blood picture was detected only when other toxic effects were visible. A dose which gave only a slightly toxic reaction was seen in one animal to cause a slight anemia without any change in the white cell count. Doses which became extremely toxic caused a striking alteration in the blood picture. Complete studies were made on only two animals of this type, but casual observations indicated that other animals receiving toxic doses followed the same course. There was a gradual development of anemia, accompanied by a rather gradual but distinct leukocytosis during most of the period of treatment. However, toward the end of treatment the leukocyte count fell, and with the extreme cachectic condition a sudden leukopenia occurred (1,000 WBC).

The fact that estrogen treatment readily causes cervical metaplasia in the monkey (13, 5), and cervical cancer in mice (7) suggested the female genital tract as the most probable site of malignancy in the chronically treated monkeys. However, no malignant growths were observed. Marked metaplasia of the epithelium of the cervical glands occurred (Figs. 25 to 35), and sometimes the glands showed a strongly cystic condition (Figs. 27 and 36). The metaplastic epithelium began to develop in localized areas (Figs. 25 and 26) at the base of the normal columnar epithelium and pushed up to displace it with a stratified epithelium (Figs. 29, 30 and 34). This metaplasia usually involved only the tips of the glands (Figs. 31 and 32), but occasionally became more extensive (Figs. 28 and 33). The cervical glands sometimes became so hyperplastic that the condition might almost be termed adenomatous (Fig. 35). However, nothing resembling cervical carcinoma developed even though the cervices of 4 monkeys were subjected to repeated trauma (cauterization, biopsies and suturing with a large surgical needle) during 6 months to a year and a half (Fig. 37). After prolonged

treatment the myometrium became quite fibrotic and often contained numerous hyalinized areas, resembling to a great extent the old age changes which occur in the uterus. The endometrial glands at first showed the typical estrogen response, but after the exhaustion phase of the uterus had set in (following prolonged treatment) the glands were few in number and were long and straight with marked hyalinization around the deep portions of the glands. The vagina was usually quite hyperplastic, and epithelial bulbs were frequently found along the basal layer (Fig. 38).

Although several animals (Table I) received very high doses of estrogen, over extended periods of time, there was no evidence of pathological change in the mammary glands. This observation held true even though 3 animals had the estrogen injected directly into the gland in an attempt to increase the local concentration of the hormone. As has been shown before (8), full development of the mammary glands can be obtained in the monkey with estrogen alone. This requires 5 to 6 months of treatment. In the chronically treated monkeys secretion was present in the glands regardless of whether the animal's ovaries were present or not. Occasionally cystic dilation of the ducts was seen. The metaplasia of the lactiferous ducts which occurred when carcinogenic hydrocarbons were given in conjunction with estrogen will be discussed under the effects of the carcinogens since these effects also occurred when the carcinogenic hydrocarbons were given alone.

Methylcholanthrene.—Since the most profound tissue changes were obtained with methylcholanthrene, the lesions caused by this substance will be discussed first. As was described in the section on material and methods, the carcinogenic agent was applied as directly to the tissue as possible. There were apparently no toxic effects of the methylcholanthrene as such, even when massive doses were given orally or intravenously. When the agent was painted on the skin, hyperkeratinization always occurred, the early tissue changes being detectable within a week. At first there was a gradual thickening of the epithelium which was due principally to an increase in the thickness of the cornified layers, particularly the sloughing stratum disjunctum. There was a gradual atresia of the sebaceous glands and a thinning of hair with the eventual formation of cystic sebaceous glands and hair follicles filled with sloughed cornified epithelium (Figs. 13, 19 and 22). The basal layers of the epithelium proliferated very rapidly and frequently formed broad pegs of epithelium (as shown in Fig. 20 after benzpyrene treatment) or malignant-looking branching down-growths (Fig. 21). The latter occurred following the extreme hyperkeratinization and ulceration. The surface epithelium at other times appeared to be extremely disorganized. These changes have never been observed to go further than is illustrated in Fig. 21, and they clear up after the application site is moved. There was an increase in fibrous connective tissue in the subcutaneous layer which became the primary tissue reaction when the carcinogen was injected subcutaneously in oil (Fig. 18; compare with Fig. 17).

The painting of areas with carcinogen in either benzene or methyl salicylate usually caused an extensive dermatitis. Painting with benzene alone produced only a mild superficial dermatitis. It was almost impossible to keep these areas aseptic and some of the extreme inflammation seen must have been partially due to infection. It is impossible, however, to separate the two effects. Inflammatory lesions were commonly produced and were followed by the formation of granulation tissue and scabbing, which, combined with the sloughed epithelium and dried carcinogen, formed warty excresences (Figs. 11 and 12). Removal of the latter left a very hyperemic, easily bleeding, pitted surface which usually continued to ooze a serous exudate. The whole area eventually became walled off by coarse collagenous connective tissue fibers, and the thickened, scarred surface became relatively avascular. This, together with the hyperkeratinization, probably made the area relatively impervious to the carcinogen.

It was impossible to separate the effects of infection from the effects of the carcinogen when the carcinogens were injected in oil, particularly when ulcerations occurred since here again the treating agent was not aseptic. The general effect of the injection of methylcholanthrene in oil was to produce a mild granulation at the site of injection so that often a definite mass could be palpated within 7 days after the first injection of the carcinogen. These masses continued to enlarge gradually and after a few months often became more than 3 cm. in diameter. The latter were usually simply granulomatous masses (Figs. 39 to 43), but frequently, with continued injections, they became inflamed and often broke down and ulcerated, making it necessary to change slightly the injection site. Usually, however, there was a more diffuse reaction which involved an increase in the number of collagenous connective tissue fibers in the subcutaneous tissue, with small localized areas of lymphocytic infiltration. There were occasional areas of acute inflammation (polymorphonucleated cells), with numer-

ous macrophages, some of which had phagocytized crystals of the carcinogen.

Some of the first pellets that were implanted ulcerated out of the subcutaneous tissue. However, with proper care in making and inserting pellets of methylcholanthrene, the only reaction seemed to be the formation of small granulomatous nodules which soon regressed to form firm connective tissue capsules about the pellets (Fig. 45). There was an appreciable inflammation between the connective tissue capsules and the pellets (Fig. 48) which also was often present in certain areas outside the capsule (Figs. 46 and 51). This condition persisted for several months. There was no evidence that the pellets were so effectively walled off that this carcinogen could not get out to the tissues. Crystals of methylcholanthrene placed in the omentum usually became surrounded by basophilic fibroblasts. Oral and intravenous administration was without effect. Gross lesions which occurred when methylcholanthrene was injected and painted on the ear are shown in Fig. 7. These lesions never became malignant, and when injections and painting were stopped they eventually healed, even though a pellet was placed in the same region.

When the breasts were treated with methylcholanthrene, numerous granulomatous masses formed, many of which became inflamed, broke down and ulcerated. The ulcerated areas were eventually healed by extensive formation of scar tissue (Figs. 2 and 3). Often the nipple became undercut by fibrous connective tissue so that presumably the lymphatic drainage was interfered with, and the nipple became extremely edematous, swelling to 4 or 5 times its normal size (Fig. 2). A great increase in the amount of collagenous connective tissue usually occurred in the subcutaneous tissue of the nipple. The other cutaneous and subcutaneous changes were the same as the general reactions described above except that there was an accumulation of hyaline material. There was usually a very marked metaplasia of the lactiferous and interlobular ducts (Fig. 13). The most extreme metaplasia of the mammary ducts occurred when the carcinogen was injected directly into the lactiferous ducts. This was evidently due to the carcinogen since injection of the oil solvent alone did not produce these changes in the breast. The intralobular connective tissue appeared quite normal following injection of the carcinogen, but the interlobular connective tissue was very dense, and usually there was almost a complete absence of fat in the interlobular spaces. However, these changes never progressed any further, even when treatment was continued up to 8 years. Oil cysts were found in the control breast which was treated with sesame oil, and lymphocytes and macrophages were also frequently present.

When the carcinogen was instilled into the cervix or injected into the uterine cavity, there was a distinct metaplasia of the cervical glands and hyalinization of the cervix that appeared to be identical with the metaplastic changes in the cervices of estrogen-treated animals, with the possible exception that changes were not as marked. Injections into the wall of the cervix or beneath the vaginal epithelium caused the same inflammatory changes in the tissues as were described for the skin and subcutaneous tissue. Rather large inflammatory lesions were produced (Figs. 23 and 24). The endometrial changes reflected the sex hormone levels, but there was some hyalinization which was presumably caused by the carcinogen. Hyalinization was apt to be more extreme in the myometrium which usually also showed a definitely increased fibrosis.

Dibenzanthracene.—The effects of dibenzanthracene were essentially the same as those described for methylcholanthrene. The necrosis and ulceration, with the resultant scarring, was certainly as great with either painting or injection (Figs. 1, 8 and 10). However, it did not seem that the deeper tissues were as greatly affected. The scarring from applications to the breasts usually resulted in interference with the lymphatic drainage, with the result that the nipple became edematous (Fig. 1). The metaplasia of the alveolar ducts was not as great as with methylcholanthrene, and there was usually an appreciable amount of fat in the gland. The general diffuse inflammation (Fig. 49) was about the same, and collagenous fibers were very numerous. The lesions around the ears (Fig. 4) were very distinct. The single animal that received pellets under the serosa of the stomach showed no gross changes. There were the usual fibrous connective tissue capsules around the pellets of carcinogen.

Benzpyrene.—Benzpyrene caused much less fibrosis than did methylcholanthrene and dibenzanthracene. Even so, palpable nodules were detected as early as 7 days following the first treatment with the carcinogen. The reaction was essentially the same as that described for the other two carcinogens. The difference seemed to be that the fibrous reaction was not so great, although in some cases extreme, yet well organized, fibrosis was present (Fig. 44). See Figs. 1-3 and 9 for gross changes in the breasts, and Figs. 5 and 6 for gross changes in the skin of the ears. Hyperkeratinization and the subsequent dermal changes occurred (Fig. 20).

There was probably somewhat less necrosis and ulceration, but both chronic and acute inflammation were present in the subcutaneous tissue. Scar tissue was quite evident. The inflammatory reaction in response to implantation of benzpyrene pellets was not as great (Figs. 47 and 50) as described for methylcholanthrene, but otherwise the capsule formation was the same. Metaplasia of the lactiferous ducts of the mammary glands (Fig. 15) occurred quite readily, with prickle cell formation and piling up of epithelium, and occasional formation of intraductal papillomas which, however, never gave any indication of malignancy. The terminal ducts were relatively unaffected (Fig. 14), and the alveoli seemed to be actively stimulated by benzpyrene (Fig. 16).

Several of the subcutaneous nodules resulting from benzpyrene treatment were extremely well vascularized (similar to Figs. 41 and 42 following methylcholanthrene treatment) and occasionally contained lymphoid nodules. One striking difference from the response to treatment with the other carcinogens was the frequent occurrence of brown pigmentation in the skin of the areas treated with benzpyrene. Sometimes this was temporary and at other times it remained until the area became extensively scarred.

Although benzpyrene was placed in the uteri of 7 monkeys, evidence of cervical metaplasia was seen in only one animal, which was also on chronic estrogen treatment. The principal reaction in the uterus was a general fibrosis of the myometrium The endometrium was usually compact, with short straight glands, if the host had been castrated.

Combination of treatment.—As may be seen in Table I, 7 monkeys received estrogen and methylcholanthrene, 9 received estrogen and benzpyrene, one received estrogen and dibenzanthracene, 6 received methylcholanthrene and benzpyrene. Four animals from the last 2 groups received all 3 carcinogenic hydrocarbons. The reactions to the carcinogenic hydrocarbons were purely local and were independent of any other carcinogen applied to another site. Therefore, the effects of the various carcinogenic hydrocarbons can be compared in the same animal, as for example, by treating one breast with benzpyrene and the other with methylcholanthrene or by treating one with dibenzanthracene and the other with benzpyrene, etc. A comparison of the effects of the various carcinogens on similar organs in the same animal confirms the finding when different animals are compared. The fundamental action was the same with all of the carcinogenic hydrocarbons. Methylcholanthrene caused the most pronounced changes; dibenzanthracene was almost as effective, and both produced more fibrotic changes than did benzpyrene. There was absolutely no evidence of any enhancement of the reaction when more than one carcinogen was present in the same animal.

Estrogen gave a systemic as well as a local effect in the doses employed. It is interesting that while estrogen and carcinogens were found to have similar effects on certain structures in the monkey, a combined treatment did not seem to give either an additive or an augmentive effect. Estrogen stimulated normal alveolar growth of the mammary glands without metaplasia of the ducts. Methylcholanthrene or benzpyrene caused a marked metaplasia of the ducts but only a moderate alveolar reaction, which was typified by a relatively low epithelium, yet there was a certain amount of secretion of an amorphous staining substance. With the combined injections the metaplasia of the ducts was the same as with methylcholanthrene or benzpyrene alone, and the alveolar development could not be distinguished from that produced by the estrogen alone. In the uterus, where estrogen, methylcholanthrene and dibenzanthracene all cause metaplasia of the cervical glands, and fibrotic changes in the rest of the uterus, the combination of estrogen and methylcholanthrene or estrogen and dibenzanthracene produced no greater effect than could be obtained by estrogen alone. Since it was necessary to apply the carcinogenic hydrocarbon directly to the uterus, the presence of the carcinogen could usually be detected by the inflammatory reactions. Otherwise, it is doubtful that its effect could have been distinguished from that of estrogen in histological preparations of the tissues.

Animals still alive.—Four monkeys are still alive and in good health after from 5 to 10 years of treatment with estrogens and carcinogenic hydrocarbons. In the 5 years since pellets of carcinogens replaced other methods of application the extreme hyperkeratinization and the ulcerated lesions have completely cleared up, leaving a great amount of scarring where the ulcers were located. The larger fibrotic masses and papillae are still present but are somewhat smaller in size and less firm in consistency than when injections and paintings were stopped. The animals with the extreme reactions shown in Figs. 2, 3, 6 and 7 are still alive and, despite the fact that pellets of carcinogens have been present in the tissue around the lesions since these pictures were made, more than 6 years ago, all lesions are healed, and the skin has taken on a healthy appearance. The general fibrosis and the grandulomatous nodules never completely dis-

appear, but they gradually take on the feel and texture of the surrounding tissue. On careful examination the numerous scars can be seen, and some fibrosis can be palpated along with the small subcutaneous nodules. The latter are appreciably smaller than when treatment was changed and have been in this condition for some time. Small papillae are also present on the skin, but they are of a soft, pliable nature in contrast to the very hard, coarse, and brittle condition observed at the time injections and paintings were discontinued and the pellets implanted. Biopsies of the old fibrosed areas show that the coarse collagenous fibers become less and less obvious with time, despite the presence of pellets of carcinogen in the nearby tissue. There is a gradual accumulation of fat in the formerly granulomatous nodules, so that after 3 or 4 years fat may replace a large portion of the nodule. It becomes extremely difficult to tell these areas histologically from the normal *tela subcutanea*. The papillae are very similar to the normal skin of the area and seem to represent a mechanical inability of the skin to return to its original shape rather than any altered histology. The reactions to newly implanted pellets continue to be the same as those already described for each of the carcinogens. Pellets which have been present for long periods of time show only the connective tissue capsule.

DISCUSSION

It is evident from the results just described that malignant tumors have not been produced in the monkey by chronic treatment with the female sex hormones and the 3 most commonly used carcinogenic hydrocarbons, methylcholanthrene, benzpyrene and dibenzanthracene. These findings are made more striking by the fact that the carcinogenic compounds have been supplemented in some animals by numerous and extreme inflictions of trauma. Moreover, periodic acute inflammation has been superimposed upon the chronic inflammation that occurs at the sites of application of the carcinogen. This has been especially true when the substance was injected subcutaneously in sesame oil (Fig. 49).

Negative results are usually considered rather unsatisfactory since their validity is limited in scope. They apply much more rigidly to the experimental conditions than do positive findings. Therefore, a critical analysis of the extent to which the conditions of the present study adhere to the generally accepted requirements for the induction of malignancy is advisable. Information on all types of experimentally produced tumors indicates that the following factors are of importance: age of host, period of treatment, proper treating agent, method of application, and dosage. The actual age of these animals was not always accurately known. However, about one-third of the group of monkeys were at the age when the menstrual cycle was just being established (3 to 4 years), and about half were well developed, sexually mature animals whose ages were estimated to be at least 8 years. Of the mature animals, 4 were treated for from 7 to 10¾ years, which would bring their ages up to rather more than the average life expectancy (16 years) of monkeys reared in captivity or under laboratory conditions. Therefore, it can be assumed that an adequate age range has been covered and that treatment has been continued over a sufficiently long period of time (up to 10¾ years). Also, since treatment was continued over a period from adolescence to old age, it is to be assumed that any relationship of malignancy to age would be covered. It is true that only 4 of the animals meet the extreme of these two factors. However, there are 9 other animals, treated from 3 to 6 years, whose distribution over the age range greatly lessens the possibility of having failed to treat the animals at the right time.

It would seem that the requirements concerning methods of application have been met quite adequately since almost all the methods that have been employed to obtain cancer in the smaller laboratory mammals have been used. The carcinogens have been applied to the skin in benzene (in one experiment in methyl salicylate; they have been injected intradermally, subcutaneously and intramuscularly; and have also been injected into the mammary glands, intracervically, into the uterine cavity and into the myometrium. They have been injected in solution in sesame oil and olive oil, and in normal saline suspension. Crystals of the carcinogens have been placed in the various viscera surgically, and pellets of 12 to 15 mgm. have been implanted subcutaneously and into the various organs most subject to malignancy in man. Intravenous and oral applications have also been made. Injection directly into the lymphatic system was not done because of the difficulty of execution, and it was felt that this route would be adequately covered by the other methods of application. Nor was the carcinogen put into the brain or cranial cavity, since it was felt that if lesions of the type produced in other areas developed, they would kill the animal and it would not be known whether neoplasms would have eventually appeared.

Dosage does not seem to be as important as other factors, except in the case of estrogens, which

must be given in fairly large doses over prolonged periods, as has been done in these experiments. The carcinogenic hydrocarbons were applied so that, with minor exceptions, each animal received from 2 to 6 mgm. weekly until the animal came to autopsy. Thus several animals received a total of around a gram of methylcholanthrene plus a half gram of benzpyrene or dibenzanthracene. The largest doses given were 1.615 gm. of methylcholanthrene and 700 mgm. of benzpyrene to one animal; and 380 mgm. of methylcholanthrene, 430 mgm. of benzpyrene and 5.323 gm. of dibenzanthracene to another. There is evidence from tissue culture studies (3) that too high a concentration of carcinogenic hydrocarbon retards the growth of cancer cells. However, the slow rate of absorption of the pellets of carcinogen and the fact that there has been no evidence of inhibition of cellular activity in the immediate vicinity of the pellets or crystals of carcinogenic hydrocarbons, even when granulomatous changes were occurring, seems to rule out such an interpretation as the cause of the failure to induce cancer in the monkey.

The results of painting the carcinogens on the skin are in agreement with those obtained by Bonne, Lodder and Streef (2) following the painting of coal tar on the skin of the Javanese monkey (*Macaque cynomolgus*). In the latter experiments the animals received cutaneous applications of tar twice weekly for periods up to almost 2 years. Hyperkeratinization, necrosis, ulceration and proliferative growth such as occurred in the present experiments were readily produced. On the basis of histological structure the authors diagnosed these lesions as epithelial carcinomas but pointed out that they never metastasized. Moreover, the lesions regressed following cessation of the tar painting and often showed regressive changes while painting was being continued.

A comparison of the action of the carcinogens in the monkey with those in the lower mammals indicates that the lesions obtained in the monkey, especially the relatively unorganized proliferation of the epithelium and the marked metaplasia of certain epithelia, are very similar to the precancerous lesions in other animals. It seems that in the monkey the proliferative stimulus can be induced in appreciable intensity, but the tissue never completely loses its power of organization and cannot progress into the truly neoplastic state. The latter may be the reason why we are able to see differences in the response to the three carcinogenic hydrocarbons, an observation which apparently has not hitherto been reported. The essential reaction to each hydrocarbon was the same, but benzpyrene caused less fibrotic change than did methylcholanthrene and dibenzanthracene. If the primary lesions had gone on to the neoplastic state, these differences would not have been so readily seen; on the contrary, because of the failure to reach the neoplastic condition, one could not determine in the monkey whether early regressive changes are a prerequisite to cancer formation, as evidence from lower animals seems to suggest (9, 11).

It has been shown in the lower mammals that action of the carcinogens does not necessarily involve any irritation or inflammatory reaction (9). The findings on the monkey are probably in agreement with this, despite the fact that inflammation to a greater or lesser degree is usually present. Epithelial metaplasia and a certain amount of fibrotic change can occur without any inflammatory reaction. It seems clear, moreover, that the reaction to the carcinogen is something more than a foreign body reaction, even though the response of the deeper structures is a general, diffuse fibrosis or the production of a nodule of typical granulation tissue. These granulomas pass from the early epithelioid cell and leukocyte state to a condition where immature fibroblasts predominate and finally relatively coarse collagenous fibers appear. Infections certainly play a prominent role in the extreme ulceration observed. However, when the direct source of the trauma is removed, the ulcerated areas slowly heal, even though the carcinogen is applied in another area or placed as a pellet in the nearby un-ulcerated tissue. The healing process and scar formation are identical with the classical description of these processes (12). The failure of the general fibrosis and the gross nodules to completely disappear is due to mechanical factors. The tissue composing these areas is, however, replaced by as nearly normal tissue as the mechanical factors will allow. The fact that there is a minimum of reaction to implanted pellets, if care is used in preparing and inserting them, also indicates that the inflammation and ulceration are secondary reactions.

The findings regarding the response of the monkey to chronic estrogen treatment are in complete agreement with those of Engle, Krakower and Haagensen (4). At the time the present experiments were begun in 1936 the concept, now supported by many investigators, that the role of estrogen in the production of mammary cancer is only to develop the mammary gland to the state at which other factors, if present in sufficient force, can cause neoplastic growth, was not in existence. Since mammary cancer has not been reported in breeding female Rhesus monkeys, this concept would have

argued against the chance of success from attempts to induce mammary cancer by the administration of estrogen, unless, possibly, the estrogen were given in combination with carcinogenic hydrocarbons. In view of the fact that the hydrocarbons have now been shown to be ineffective themselves, the results obtained are to be expected.

The incidence of tumors in monkeys can only be roughly guessed. Ratcliffe (13) has reported that at least 8 tumors of all types occurred in the primates of the Philadelphia Zoological Gardens during the period from 1901 to 1932 but that this incidence was lower than for any other order of mammals. Only 2 of these tumor-bearing animals belonged to the genus Macaca and none were found in the Rhesus monkey. However, Bagg (1) has described an osteogenic sarcoma, and Engle and Stout (6) a prostatic carcinoma, in the Rhesus monkey (*Macaque mulatta*). The low incidence of tumors in primates kept in zoological gardens (13) as compared to other mammals is somewhat offset by the fact that the primates had the shortest average exhibition period. The tumor bearers among the primates had an average exhibition period (111.8 months) which was more than 6 times the average for all the primates. Among the family Caropithecidae (the old world monkeys), the average exhibition period for tumor-bearers (124.3 months) was about 8 times that for all animals in this group. These findings strongly indicate that monkeys are so susceptible to the common diseases of man that they seldom live to the tumor-bearing age. This is particularly true of the monkeys in the zoological gardens, at present our main source of data on this subject, since they are continually exposed to crowds of people. The cost of keeping monkeys under laboratory conditions has usually prevented studies on old monkeys, except as they may occasionally come into the laboratory as recognized old animals. It is also unlikely that monkeys live to a very old age in their native wild condition. These factors make it particularly difficult to determine anything about tumor incidence, yet the fact that the occasional tumors that are found are of quite diversified types, makes it seem probable that the monkey is not particularly immune to cancer in the hereditary sense. Therefore, one is led to suspect that the monkey has a very good protective mechanism against the carcinogenic hydrocarbons. The fact that the so-called precancerous lesions occur at the site of application, that the carcinogen when in a vehicle which is painted on the skin or injected by various means is more effective than when introduced as a pellet or dispersed crystals, and that the carcinogens even in tremendous doses have no systemic effect, all suggest that such a protective mechanism is in operation. The systemic protective mechanism is apparently much more effective than the local, yet the fibrotic changes around the applied carcinogens are extremely limited. This protective mechanism can hardly be explained on the basis of a simple walling off of the carcinogen because histologically this reaction is no more marked than it is with estrogen, where the systemic effect is strongly evident, or than in the mouse where the carcinogens are highly effective. Moreover, the carcinogens gradually disappear from the pellets, indicating that they are being absorbed. It may be that they are destroyed as rapidly as they are absorbed and, of course, one would link such a phenomenon with the liver. It is of interest, therefore, that the livers of monkeys, getting even the chronic treatments, show little if any impairment.

SUMMARY

Fifty monkeys received treatment with estrogens and 3 carcinogenic hydrocarbons (methylcholanthrene, dibenzanthracene and benzpyrene) for periods up to 10 years. Almost all organs and tissues that frequently become cancerous in the human being were treated, including the abdominal and pelvic viscera. The breasts received the most chronic treatment, although multiple sites were used in all animals. Carcinogens were also injected intravenously and administered orally.

Methylcholanthrene and dibenzanthracene caused more pronounced local reactions than did benzpyrene. Following injections in oil these consisted of firm inflammatory or non-inflammatory fibrotic masses, areas of granulation tissue and encapsulated cysts of the injected material. Following painting on the skin, they consisted of hyperkeratinization, alopecia, cystic hair follicles, cystic sebaceous glands and papillomatous masses or wart-like excrescences. Pellets became encapsulated by dense fibrous connective tissue surrounded by numerous lymphocytes and phagocytes. This reaction sometimes progressed until firm non-inflammatory fibrotic masses were produced, but usually the capsule gradually became indistinguishable from that surrounding any foreign material.

The lesions usually became infected and ulcerated, but if the injection site was changed, they slowly healed with typical scar formation. Sometimes there was disorientation of the tissues near the ulceration with marked papillary downgrowth of the surface epithelium. The general fibrosis consisted of large well differentiated collagenous fibers; the non-ulcerative tissue masses were typical granu-

lomas with varying amounts of chronic or acute inflammation.

Metaplasia of the mammary ducts and of the epithelium of the cervical glands occurred with both estrogen and the carcinogenic hydrocarbons, but did not progress beyond this condition. Despite the relatively unorganized proliferation or marked metaplasia and the formation of granulomas, a true neoplastic state was never reached. The proliferative stimulus was apparently present in appreciable amounts, but the tissue never completely lost its power of organization. All lesions gradually reverted toward the normal when treatment at the site was stopped, even though pellets of carcinogen were present in the nearby tissue.

The findings are negative for cancer, but it is felt that most of the requirements of a crucial test have been met.

ADDENDUM

Since the manuscript of this article was submitted for publication, a spontaneous oral carcinoma in a male Rhesus monkey has been described by Klüver and Brunschwig (Cancer Research, 7:627-633. 1947). This led to the finding in the literature reports of two other spontaneous oral carcinomas in Rhesus monkeys (Steiner, P. E., Klüver, H., and Brunschwig, A., Cancer Research, 2:704-709. 1942; and Zuckerman, S., Proc. Zool. Soc., London, 1930, pp. 59-61.) suggesting that this is the most frequently occurring type of spontaneous tumor in the Rhesus monkey.

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DESCRIPTION OF FIGURES 1 TO 7

FIG. 1.—Breasts of monkey No. 66 after 3½ years of treatment with carcinogens. The left breast was treated with dibenzanthracene (1 mgm. in 0.1 cc. of sesame oil weekly, injected subcutaneously). The right breast received weekly injections of benzpyrene (1 mgm. in 0.1 cc. of sesame oil). It can be seen that the reaction to the dibenzanthracene is more intense than to the benzpyrene, the skin around the left nipple showing much greater proliferation. There have been areas of necrosis which are now represented by the rather marked scarring suggestive of keloid formation. There is a greater loss of hair and greater enlargement of the nipple, presumably due to interference with the lymphatic drainage of the nipple by the scarring at the base of the nipple. Fibrous tissue formation is quite marked in both breasts, with definite masses fixed to either the underlying fascia or to the epithelium. This is a chronic condition.

FIG. 2.—Breasts of monkey No. 92 after chronic treatment for 3½ years. The right breast was treated with benzpyrene (1 mgm. in 0.1 cc. of sesame oil) injected subcutaneously at weekly intervals. The left breast was painted biweekly with 1 mgm. of methylcholanthrene in benzene. Firm connective tissue masses are present in both breasts. One large mass, 1 × 1 cm. in diameter, can be seen adjacent and medial to the right nipple. The painted area is characterized by loss of hair and an epithelial reaction similar to that shown in Fig. 13. The extreme edema of the nipples is presumably due to undercutting by scar tissue which interferes with the lymph drainage.

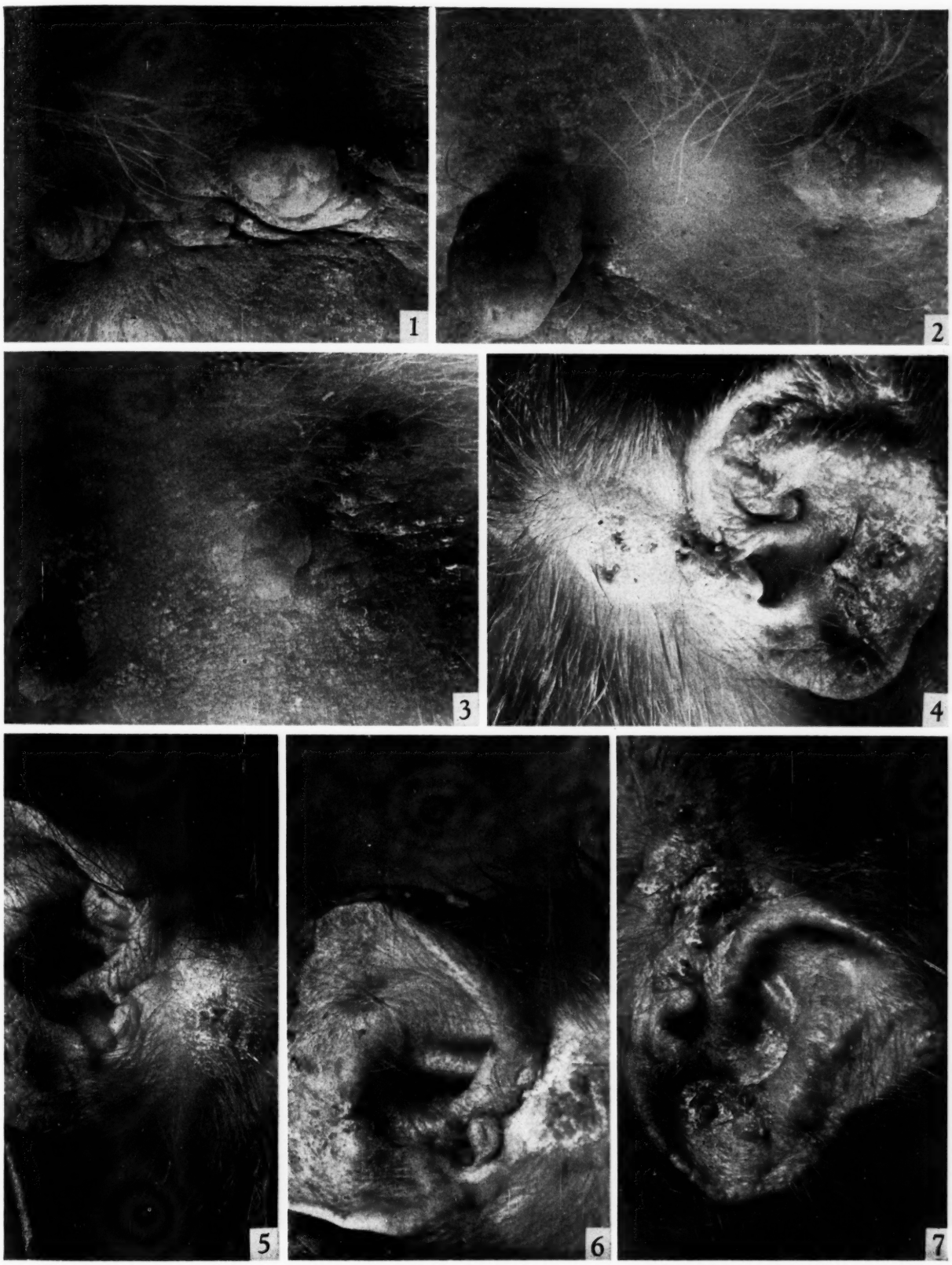
FIG. 3.—Breasts of monkey No. 705 after chronic treatment for 2 years. The right breast was injected subcutaneously with benzpyrene (1 mgm. in 0.1 cc. of sesame oil weekly). The left breast was injected with 1 mgm. of methylcholanthrene weekly and painted with methylcholanthrene in benzene twice weekly (2 mgm. weekly). Firm connective tissue masses are present in both breasts. The painting of the left breast produced thickening and scaling of the epithelium with multiple punctate areas of proliferation. The scaling of these irregular macules and papules is seen most clearly lateral to the left nipple.

FIG. 4.—Left ear of monkey No. 66. Dibenzanthracene (1 mgm. weekly) had been injected subcutaneously for 3½ years. Injections were started on the helix and antitragus of the pinna, but ulceration forced a shift to the area just anterior to the tragus. This area also ulcerated after 6 months, leaving the several sinuses seen in this illustration. The injection site was then moved to a location slightly more anterior. The mass shown in the photograph is clearly circumscribed and is made up in part of proliferated subcutaneous fibrous tissue and in part of proliferated epithelium. The old site of injection on the pinna is readily visible as a scarred, desquamated and proliferated area.

FIG. 5.—Right ear of monkey No. 66. Benzpyrene (1 mgm. weekly in sesame oil) had been injected subcutaneously for 3½ years. There is little effect at the injection site on the pinna except for a condensation of connective tissue which prevented retention of the carcinogen. Note the thickening of the anterior helix. Anterior to the tragus (the last site of injection) a well circumscribed mass similar to that described in Fig. 4 is seen. The reactions here differ from those following dibenzanthracene in that they are not so intense, and there is no tendency to ulcerate.

FIG. 6.—Right ear of monkey No. 705 which had received the same treatment and shows the same reaction as described for Fig. 5. Compare this reaction to benzpyrene with the effect which methylcholanthrene produced on the left ear of this same animal, shown in Fig. 7.

FIG. 7.—Left ear of monkey No. 705. For 2 years methylcholanthrene had been applied to the pinna of the ear and the skin anterior to the tragus by subcutaneous injection (1 mgm. weekly) and by painting (2 mgm. weekly). The first injections were at the helix and antitragus, but ulceration soon forced a change in the injection site to the anterior helix and the epithelium anterior to it. Note that not only is there a clearly circumscribed fibrous mass at the latter injection site, but there is also a marked epithelial reaction which may be due in part to the combined injection and painting. The old sites are marked by definite scars. The reaction is much more extreme than that following benzpyrene, shown in Fig. 6.



FIGS. 1–7

DESCRIPTION OF FIGURES 8 TO 12

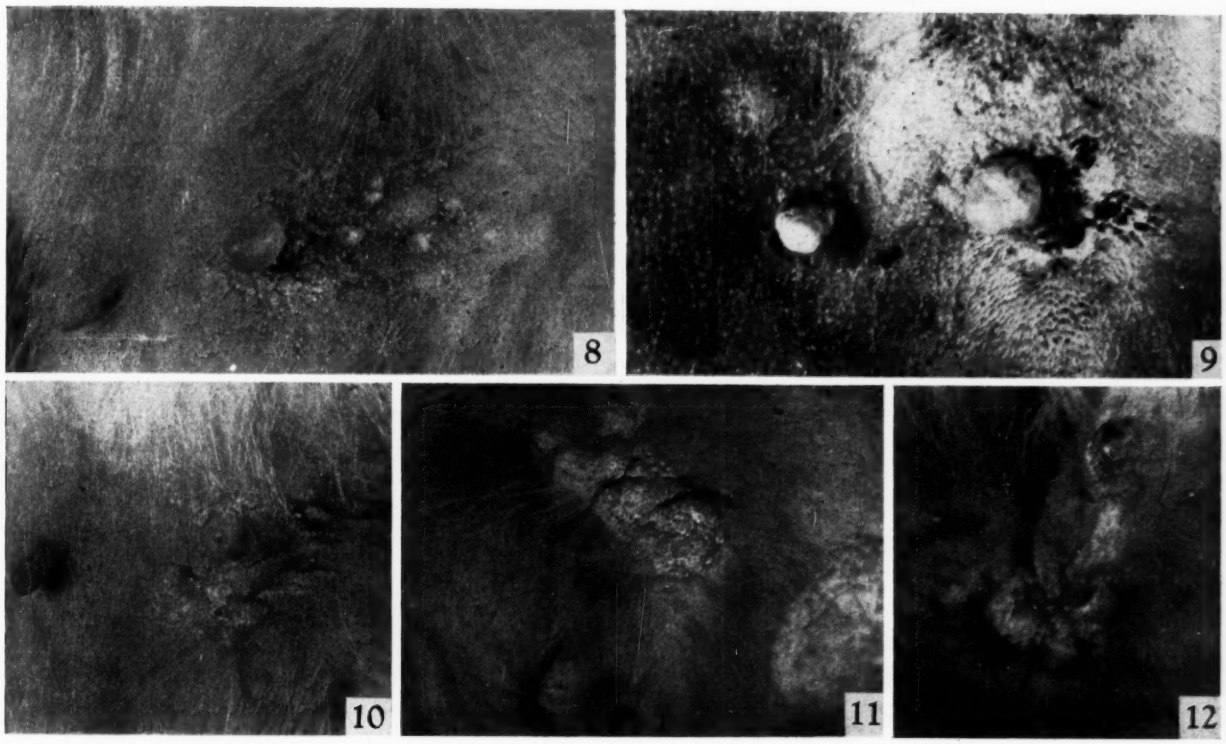
FIG. 8.—Left breast of monkey No. 10 after treatment for 1 year with approximately 6 mgm. of dibenzanthracene weekly. The carcinogen was injected subcutaneously in sesame oil. Note the ulcerated area just lateral to the nipple. Other areas of ulceration have healed with scar formation, seen among the dermal papillae. The entire area shows extreme hyperkeratinization with some of the fibrotic masses visible as larger elevated areas.

FIG. 9.—Breasts of monkey No. 61. The left breast had been injected with 2 to 4 mgm. of benzpyrene weekly for 15 months. Note the numerous ulcerated areas just lateral to the nipple which has become edematous due to scar formation at its base. There are some fibrotic masses in the connective tissue of the breast, but they are not as extreme as those found following treatment with methylcholanthrene and dibenzanthracene. This is about the most extreme reaction to benzpyrene observed in the breast region.

FIG. 10.—Breasts of monkey No. 42. The left breast had received injections of dibenzanthracene (2 mgm. weekly) for 1 year. The right breast had received control injections of oil of sesame. Note the areas of inflammation, ulceration, hyperkeratinization, and papilla formation, as well as the clearly evident subcutaneous fibrotic masses. The local effect is as extreme as with the much higher dose (*see* Fig. 8).

FIG. 11.—Male monkey No. 5 which had been injected over the sacrum with 1 mgm. of methylcholanthrene weekly. Note the papillomatous-like mass which developed at the base of the tail. Autotransplants to several abdominal sites failed to grow.

FIG. 12.—Monkey No. 19 which had been injected with 1 mgm. of methylcholanthrene weekly on the under surface of the tail or between the base of the tail and the anus. Note the granulomatous masses which have partly broken down and ulcerated.



FIGS. 8-12

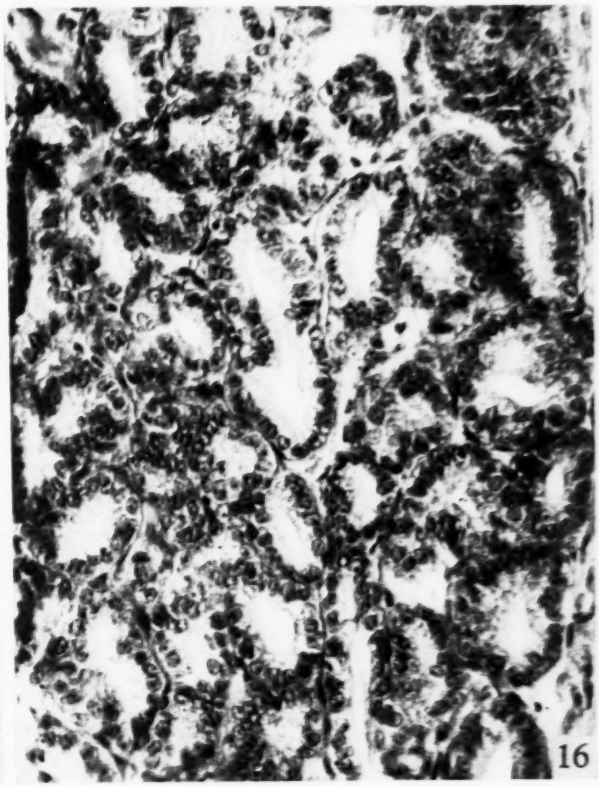
DESCRIPTION OF FIGURES 13 TO 16

FIG. 13.—Low power magnification of a section through the skin and mammary duct of monkey No. 43. Methylcholanthrene had been injected at the base of the nipple. Note the hyperkeratinization of the hair follicle and the metaplasia of the mammary ducts, seen in the center of the photograph. There is an appreciable amount of chronic inflammation in the subcutaneous tissues. Mag. × 31.

FIG. 14.—Terminal duct of the right mammary gland of monkey No. 66, showing increased connective tissue fiber formation and absence of metaplastic changes in the terminal duct. This breast had received subcutaneous injections of 1 mgm. of benzpyrene weekly. Mag. × 140.

FIG. 15.—Lactiferous duct of the breast described for Fig. 14. Note the extreme metaplasia. Mag. × 140.

FIG. 16.—High power magnification of mammary gland showing the maintenance of active alveoli by the benzpyrene. From the breast described for Fig. 14. Mag. × 280.



FIGS. 13–16

DESCRIPTION OF FIGURES 17 TO 22

FIG. 17.—Monkey No. 61. Normal skin from an area at some distance from any site of administration of carcinogen. Mag. × 100.

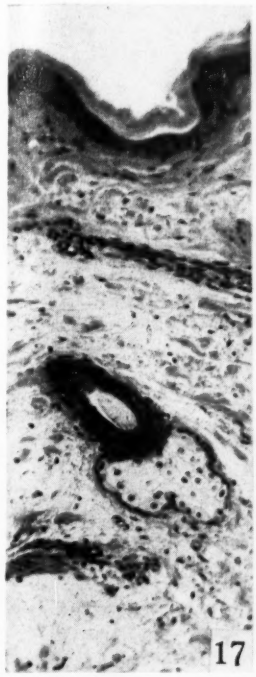
FIG. 18.—Monkey No. 145. Skin after a relatively short period of injection of methylcholanthrene. Note the increased fibrosis and the absence of hyperkeratinization or effect on the hair follicles and sebaceous glands. Mag. × 100.

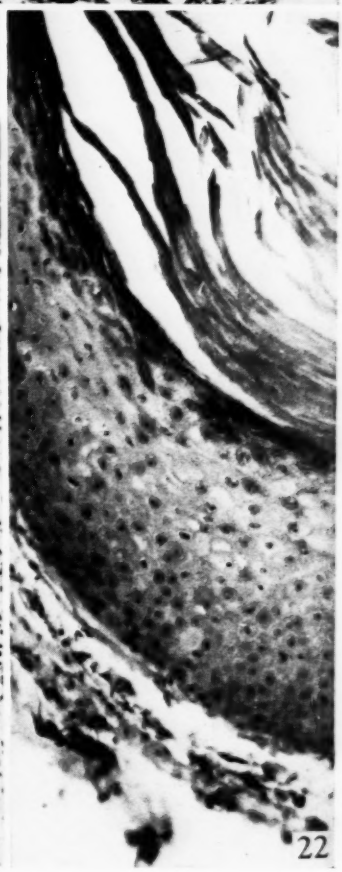
FIG. 19.—Monkey No. 43. Skin after painting with methylcholanthrene in benzene. Note the extreme hyperkeratinization of the surface epithelium which extends into the hair follicles and sebaceous glands, causing alopecia. At the extreme right center of the photograph is a cystic follicle greatly distended by sloughed keratinized epithelium. Just to the left and inferior to it is a cystic sebaceous gland which is also distended with sloughed keratinized epithelium. At the upper left is a distended hair sheath. This condition also occurs following the deeper reaction which results from injection of the carcinogen but is obtained more quickly by painting. Mag. × 60.

FIG. 20.—Skin of monkey No. 61. Broad pegs of epithelium growing down into the corium following prolonged treatment with benzpyrene. These downgrowths occurred after the necrosis and ulceration of the deeper areas described for Fig. 9. Mag. × 100.

FIG. 21.—Skin of monkey No. 96 following prolonged painting with 2 mgm. of methylcholanthrene in benzene weekly. The extreme epithelial downgrowth illustrated here occurred following a primary extreme hyperkeratinization and ulceration. Most of the surface epithelium is greatly disorganized or sloughed. Mag. × 100.

FIG. 22.—Monkey No. 66. A small segment of a cystic hair follicle to illustrate the extremely active hyperkeratinization. From the right breast described for Fig. 1. Mag. × 140.





FIGS. 17-22

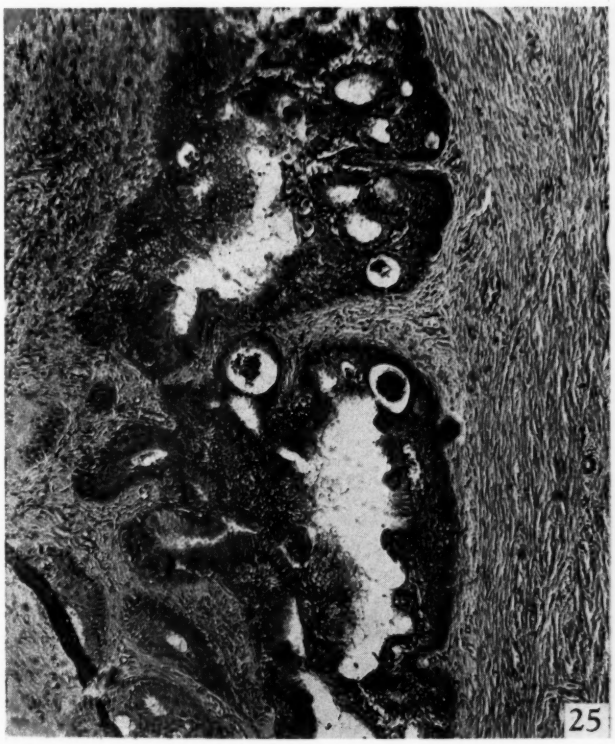
DESCRIPTION OF FIGURES 23 TO 26

FIG. 23.—Monkey No. 96. Papillary downgrowth of the cervical epithelium; 2 mgm. weekly of methylcholanthrene in oil had been instilled into the cervical canal. Extreme epithelial reaction was observed. At first this was followed by necrosis and erosion. Note the great disorientation of the surface epithelium, the marked surface inflammation and the characteristic papillary downgrowth with occasional pearl formations. Mag. × 100.

FIG. 24.—Vagina of monkey No. 96. Note the marked cornification of the vaginal epithelium and the papillary downgrowths from it. The downgrowths show a peculiar infiltration with leukocytes. Mag. × 100.

FIG. 25.—Low power view of cervical glands of monkey No. 6. This monkey had been ovariectomized and had received 0.15 mgm. of theelin daily for 6 months. Note the moderate metaplasia of the cervical glands and the pearl-like cysts in the lining epithelium. Mag. × 75.

FIG. 26.—Higher magnification of a portion of the tissue shown in Fig. 25, illustrating the beginning of metaplasia of the cervical glands. Mag. × 280.



FIGS. 23-26

DESCRIPTION OF FIGURES 27 TO 34

FIG. 27.—A sagittal section of the cervix of monkey No. 7, which had received 0.4 to 0.6 mgm. of theelin weekly for 2 years, showing adenomas and cyst formation. Mag. × 10.

FIG. 28.—Higher magnification of cervical glands showing considerable metaplasia; from the section shown in Fig. 27. Mag. × 75.

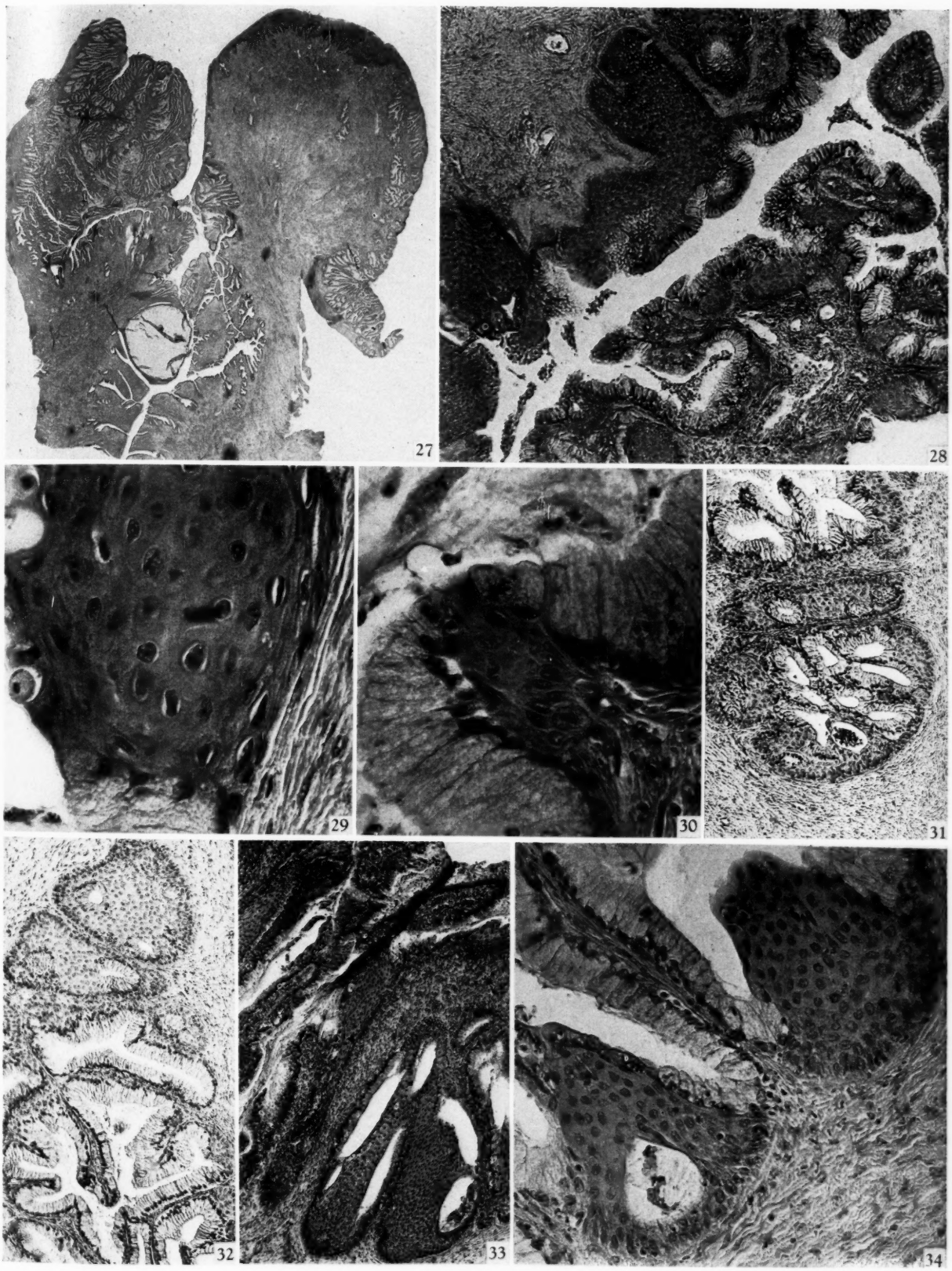
FIG. 29.—Oil immersion photomicrograph showing a small nest of metaplastic cells replacing the tall columnar epithelium of the cervical glands. The tall columnar cells can be seen at the bottom edge of the photograph. Some of the nuclei of the columnar cells still remain at the edge of the metaplastic area, which is seen at the extreme left. From monkey No. 7. Mag. × 1,500.

FIG. 30.—Oil immersion photomicrograph showing the formation of the metaplastic cells and the way in which they displace the normal cervical epithelium. From monkey No. 7. Mag. × 1,500.

FIGS. 31 and 32.—Cervix of monkey No. 90. This animal had received 0.4 mgm. of theelin weekly for 1 year and 8 months. Metaplasia is present in only the tips of the glands. The remaining portions of the glands show normal estrogen stimulation. Mag. × 95.

FIG. 33.—Cervix of monkey No. 15. This animal had received 0.4 to 0.6 mgm. of theelin weekly for 8½ months. There is appreciable metaplasia, but the cervical glands are not particularly well developed. Mag. × 75.

FIG. 34.—Monkey No. 90. Oil immersion photomicrograph showing metaplastic plugs protruding into the lumina of the cervical glands. Mag. × 500.



FIGS. 27-34

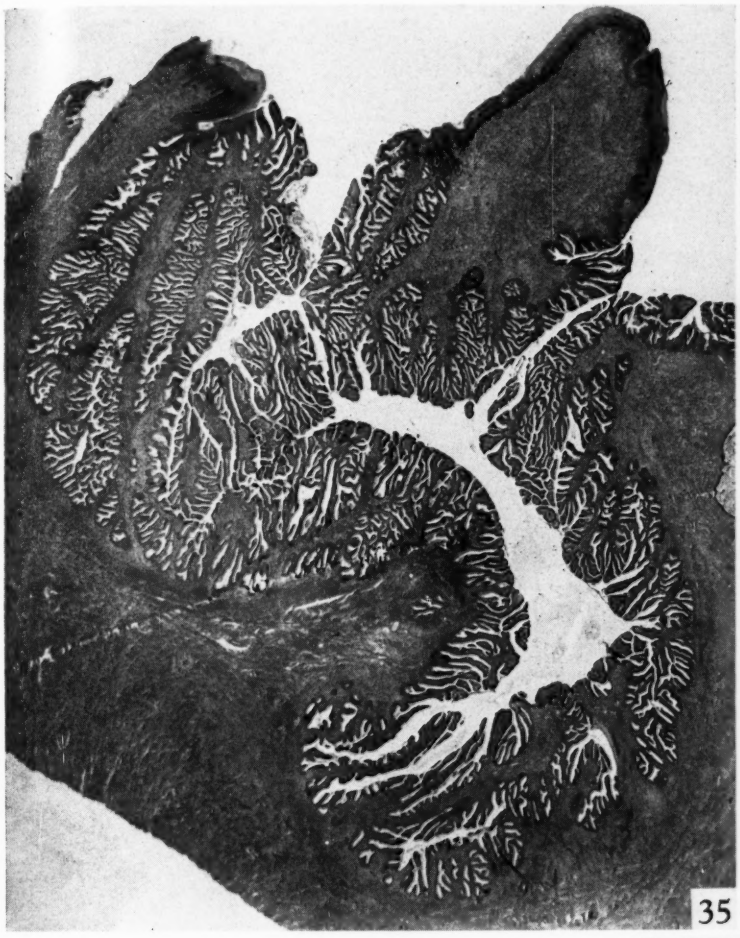
DESCRIPTION OF FIGURES 35 TO 38

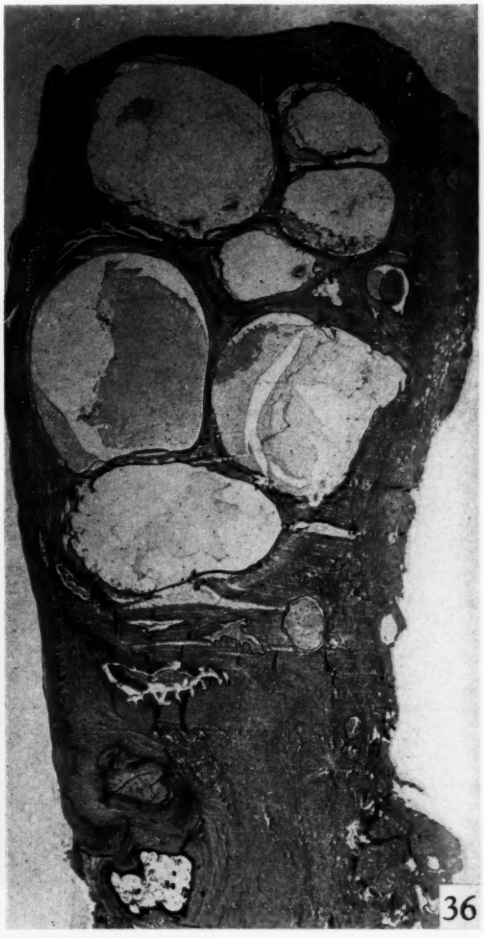
FIG. 35.—Low power photomicrograph showing the adenomatous nature of the cervix of monkey No. 90. Mag. × 10.

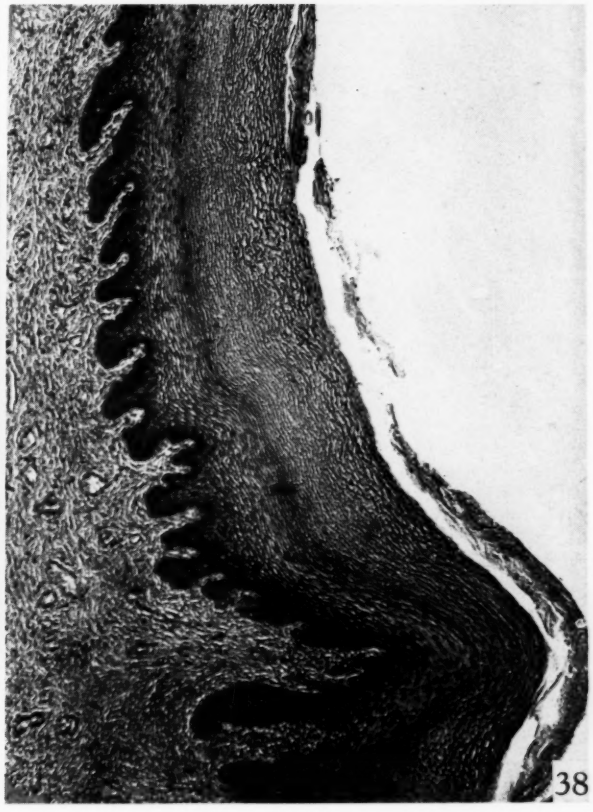
FIG. 36.—Low power view of the squamous epithelial portion of the cervix of monkey No. 90, showing the extreme cystic nature of the glands in this area. Mag. × 70.

FIG. 37.—Cervix of monkey No. 59. This animal had received 4 mgm. of theelin weekly for 15 months plus repeated cervical trauma. Note the extreme atrophy of the cervical glands. There is no epidermization. Mag. × 10.

FIG. 38.—Hyperplastic vagina taken from the same animal as shown in Fig. 37. The epithelium is fairly thick with frequent epithelial bulbs along the basal layer. Mag. × 10.







FIGS. 35-38

DESCRIPTION OF FIGURES 39 TO 44

FIG. 39.—Monkey No. 146. Early granuloma following one month of subcutaneous injection of 2 mgm. of methylcholanthrene weekly. Mag. × 140.

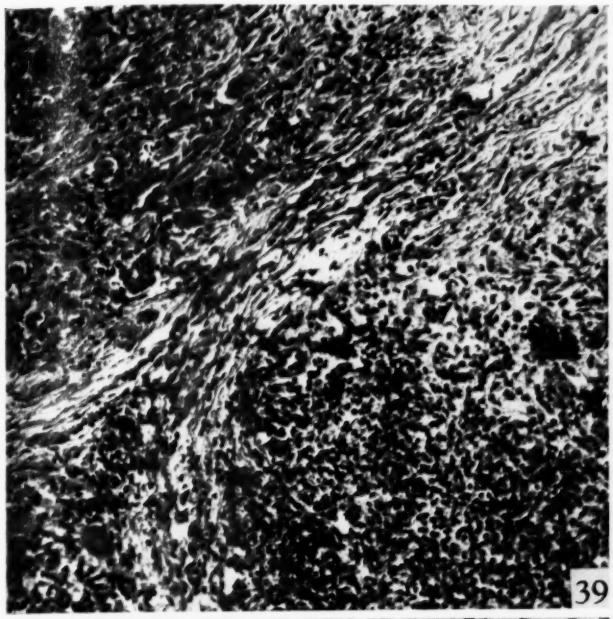
FIG. 40.—Section of a biopsy sample of the granulomatous nodule shown in Fig. 39, taken 30 days later. Mag. × 140.

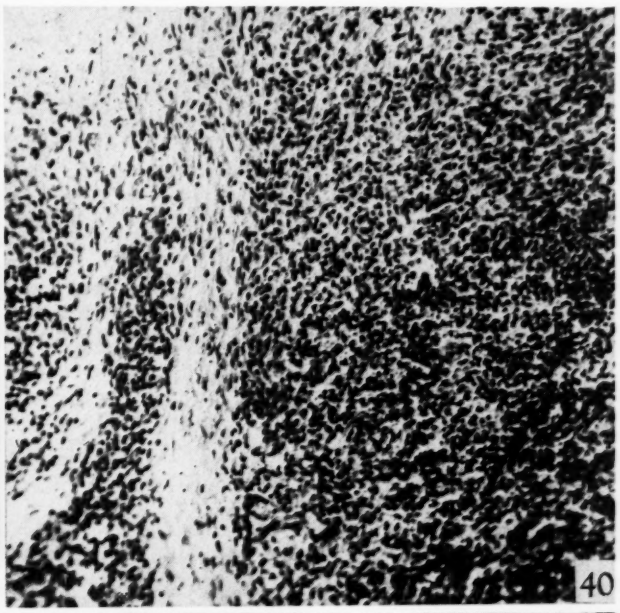
FIG. 41.—Subcutaneous nodule from monkey No. 5, the same animal as shown in Fig. 11. Note the dense, well vascularized connective tissue (after 25 months of treatment). Mag. × 140.

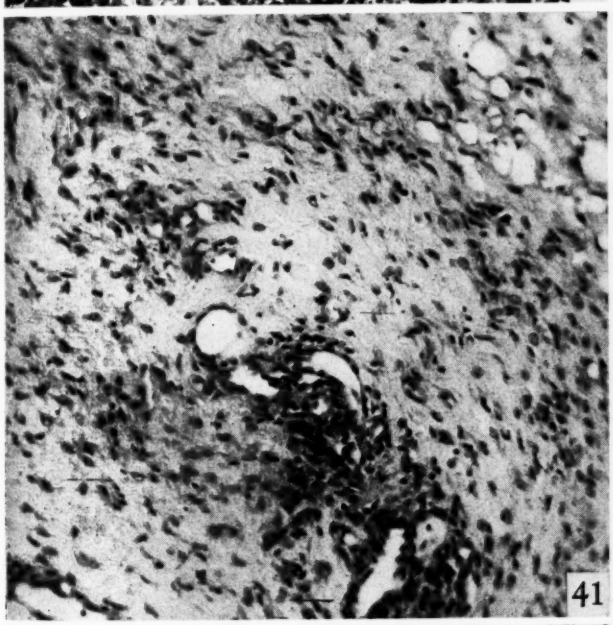
FIG. 42.—Different area from the same nodular mass as shown in Fig. 41. Note the extreme vascularity and chronic inflammation of this area. Mag. × 140.

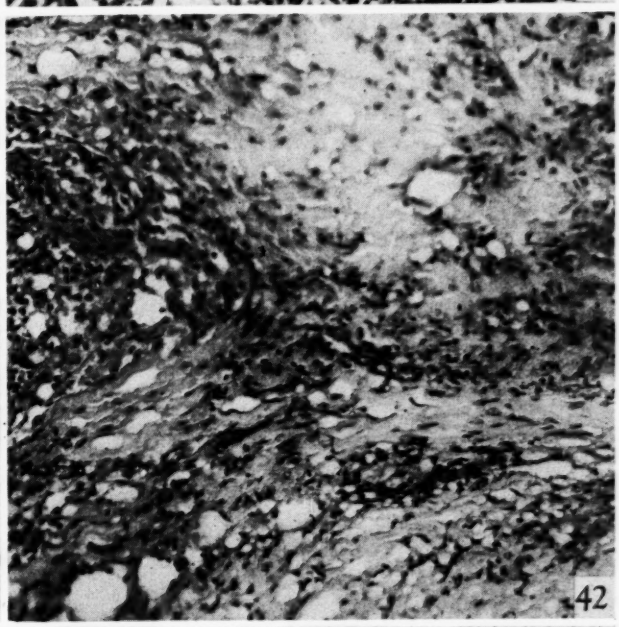
FIG. 43.—Monkey No. 19. Section of a nodular mass after 4 years of treatment with 1 mgm. of methylcholanthrene weekly. Note the dense but well organized connective tissue comprising this area, with small localized areas of chronic inflammation. Mag. × 140.

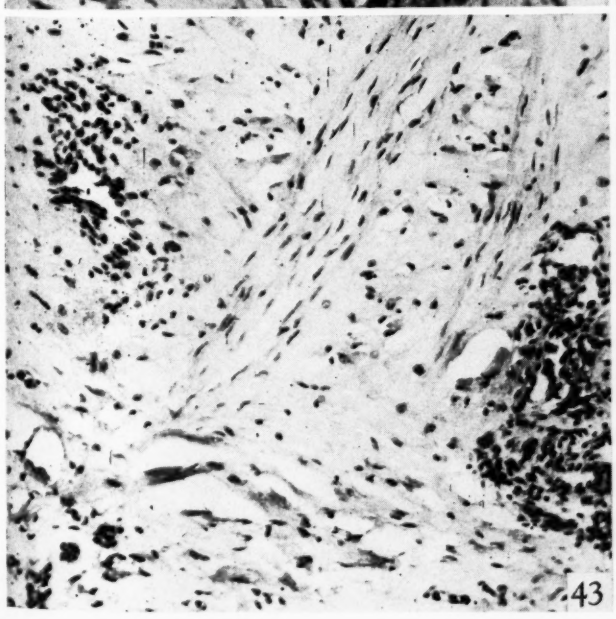
FIG. 44.—Section through a subcutaneous nodular mass from the right breast of monkey No. 66 (*see* Fig. 1) after approximately 5 years of treatment. Mag. × 140.

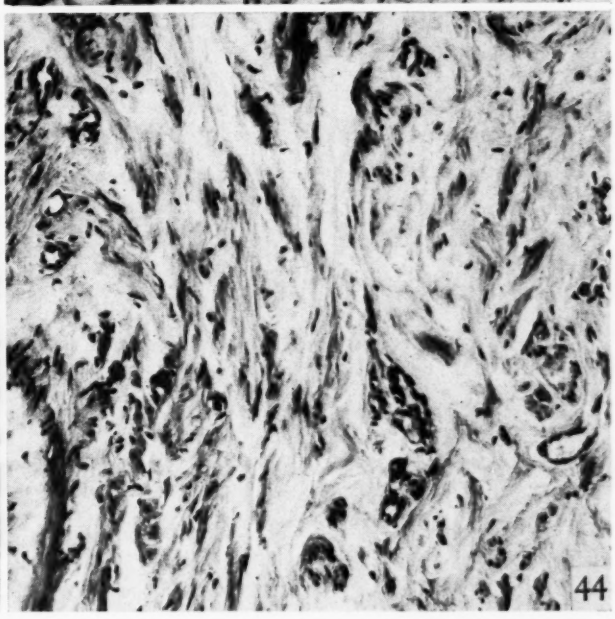












FIGS. 39-44

DESCRIPTION OF FIGURES 45 TO 51

FIG. 45.—Monkey No. 92. The capsule around a pellet of methylcholanthrene 65 days after implantation. There is a fairly wide area of inflammation between the pellet and the connective tissue capsule. In some areas there is a marked inflammation outside the connective tissue capsule (as illustrated in detail in Fig. 46), but for the most part the tissue beyond the capsule is relatively unaffected. Mag. × 40.

FIG. 46.—Enlarged section of the pellet capsule shown in Fig. 45. Note the extreme reaction adjacent to the pellet as well as the area of inflammation immediately external to the connective tissue layer. Pellet surface is at the top. Mag. × 100.

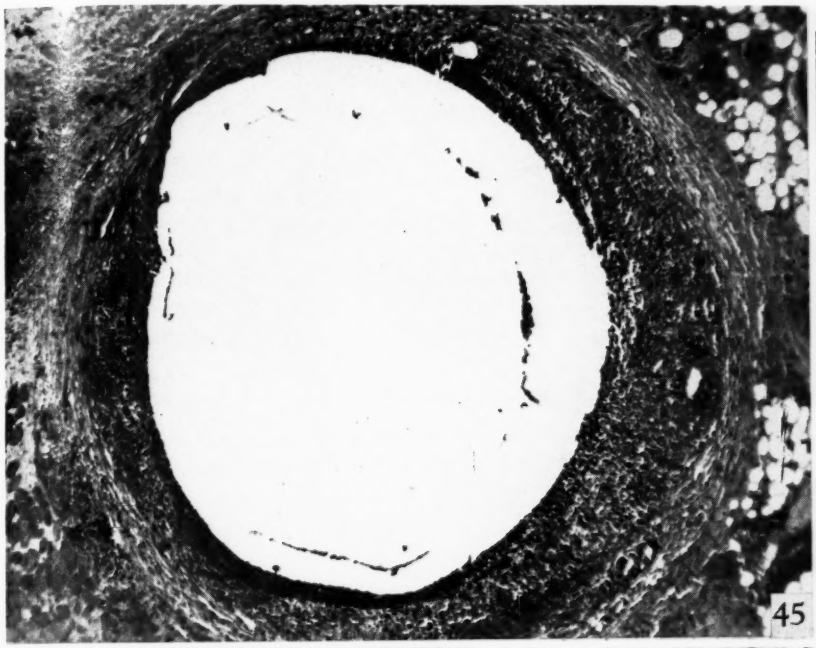
FIG. 47.—Monkey No. 92. Capsule around a benzpyrene pellet in the same animal, for the same length of time, as described for Fig. 45. There is much less tissue reaction adjacent to the pellet as well as external to the connective tissue capsule. This capsule is not as thick as the one around the methylcholanthrene pellet. Mag. × 40.

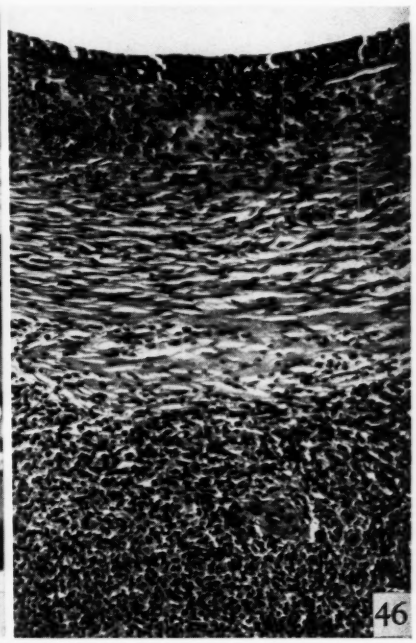
FIG. 48.—An enlarged section of the pellet capsule shown in Fig. 45 in an area where only a mild reaction extends beyond the connective tissue capsule. This photograph illustrates quite well the inflammatory reaction adjacent to the pellet. Mag. × 100.

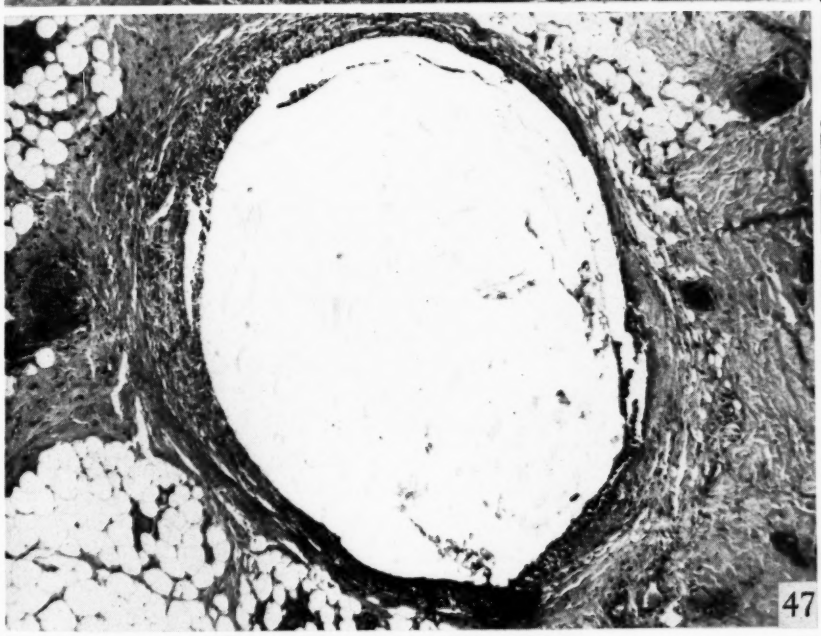
FIG. 49.—Monkey No. 42. Oil cysts in muscle following treatment with 3 mgm. of dibenzanthracene weekly. Note the chronic inflammation around the cysts. This is not markedly greater than was found around the sesame oil without the carcinogen. Mag. × 140.

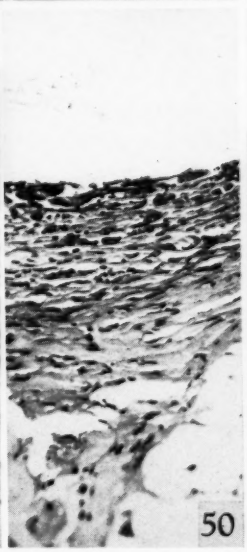
FIG. 50.—Monkey No. 92. A section through the benzpyrene pellet capsule shown in Fig. 47. Note that there is only a slight reaction adjacent to the pellet. The capsule resembles that found around an estrogen or androgen pellet. Mag. × 100.

FIG. 51.—A section of the capsule shown in Fig. 45. This shows that in certain places, even with methylcholanthrene pellets, there are only small localized areas of tissue reaction beyond the connective tissue capsule. There is, however, no evidence that the pellet is being so effectively walled off that the carcinogen cannot get at the tissues. Mag. × 100.









FIGS, 45-51

Cancer Mortality among Males and Females in Denmark, England and Switzerland

III. Cancers of Accessible and Inaccessible Sites

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It is an interesting fact that crude cancer mortalities among men and women are of almost equal magnitude, though no male organ has as high a cancer incidence as do the female breast or the uterus. This suggests the existence of factors influencing the total cancer incidence so that it may be insufficient to examine the isolated incidence of single forms of cancer. Other facts seem to strengthen this view.

Thus, Cramer found (*see* Murray [7]) that the different forms of cancer in females occurred with varying frequency in England, Holland, Switzerland and Japan although the total cancer mortality among women was about the same. This phenomenon, if true, would seem less enigmatic today, since, for obscure reasons, breast cancer increases and uterine cancer decreases in frequency with a declining birthrate.

Cramer (4) also found that with approximately identical values for cancer mortality in England and on the European continent, gastric cancer could be estimated to about 25 per cent of the total for England and about 50 per cent for the Continent. Today's figures would show similar features but less pronounced.

The cause of this apparent similarity in the incidences may be a hereditary factor favoring the development of all cancers. Thus, in Warthin's famous Family G (5) he found the same incidence of cancer among men and women, but the site varied according to sex. Consequently the present investigations into the incidence of cancer have been supplemented with simultaneous investigations into the heredity of malignant disease in such sites as the breast (6), uterus, multiple cancers, and leukemia (8) carried out in the same population and in collaboration with the University Institute of Genetics in Copenhagen.

As one carcinogenic factor may influence the frequency of more than one form of cancer it is necessary to survey cancers of all sites before examining any single form. We have in this report considered the mortality of a few comprehensive groups of cancer which presumably would be more uniform in different countries than cancers of any single site, although comparisons of the statistics for single sites must be the ultimate aim.

We have previously demonstrated (1) that about 1938 Switzerland had the highest crude cancer mortality rate in Europe for the total population and for males, and the third for females, while England was second with identical rates for both sexes. Denmark was third on the list with the highest mortality rate for females and the sixth for males. Comparisons of age distribution curves based on quinquennial age groups confirmed that these differences in the sex rate of cancer mortality were due mainly to deviation of male cancer mortality, whereas the female rates showed smaller variations in opposite direction to those of the male, thus tending to increase the international differences in the sex proportions.

It would seem that international comparisons of this kind could at present hardly be carried out on more suitable grounds. About 1937 the three countries were all prosperous, exploiting first-class medical facilities in rather concentrated populations, and showing cancer mortalities of about the same magnitude.

Furthermore, inquiries about the technic employed in the central statistical offices of the three countries have not revealed any differences of importance to the present investigations. Therefore, it seems justified to subject the material to further analysis.

The aim of this investigation is to ascertain which forms of cancer are responsible for the differences among the countries in question. Comparisons to this end will be made by means of age distribution curves, and with the methods described in our first report (1).

To avoid the effects of smaller international differences in diagnostic methods and nomenclature, we have reduced our subdivisions to only two main groups: (a) "accessible sites" in which tumors can be diagnosed with a fair degree of certainty without complicated methods, and (b) "inaccessible sites," comprising all others.

Gastric cancer has been grouped separately under "inaccessible sites." This form is outstanding among frequent cancers in its almost invariably fatal course, and consequently it greatly influences mortality statistics. At the same time the certainty of diagnosis here is smaller than for most other frequent cancers, but nevertheless it is of interest to observe how this group influences the total cancer mortalities of the three countries, and thus to test the validity of international differences in cancer mortality.

For these reasons computations have been carried out, both with and without gastric cancer as a subdivision, under cancers of inaccessible sites.

Cancers of accessible and inaccessible sites are as follows:

Accessible:

Lip
Tongue
Mouth
Tonsil
Upper jaw and ethmoidal ossa
Lower jaw
Pharynx
Nasopharynx
Salivary glands
Neck (Unspecified)
Esophagus
Rectum
Anus
Larynx
Uterus (cervix, funduss, unspecified)
Vulva and vagina
Mamma
Testis
Penis and scrotum
Other male genital organs (prostate excepted)
Skin
Head and cranium (unspecified)
Eye

Inaccessible:

Separately: Stomach
Small intestine (including duodenum)
Cecum
Hepatic flexure
Splenic flexure
Sigmoid flexure
Large intestine (Colon)
Intestine (unspecified)
Liver
Gall bladder
Pancreas
Peritoneum
Retroperitoneal tissue
Abdomen
Other digestive organs
Lung
Mediastinum
Pleura
Pericardium
Thorax
Ovary and Fallopian Tube
Other sites of female genital system
Brain, Meninges, Spinal Medulla
Other organs of Central nervous system
Thyroid gland
Kidney
Suprarenal gland
Thymus
Prostate, Bladder
"Pelvis"
Bones (jaw excepted)
"C.metastaticus"
Occult sites
Others

Sarcomata have, when specified, been grouped according to site.

Females

The differences between the age distribution curves for the total cancer mortality among women in the three countries (1, Fig. 4) are slight and not obvious in the diagram, yet they are statistically significant. The order of incidence is as follows: Denmark, England, Switzerland. If, however, the figures for gastric cancer are subtracted from the total (Fig. 1) we find that the two upper curves for Denmark and England are brought to complete correspondence, whereas the curve for Switzerland remains at a lower level.

Figs. 2 and 3, and Tables I and II, illustrate the effect of excluding gastric cancer from the group, "inaccessible sites." In these as in all our diagrams particular attention should be paid to the portions of the curves for the ages 40 to 70 years, as incidence variations in age groups comprising larger numbers of persons will carry a far heavier numerical weight than variations in the smaller age groups above 70.

The curves of Fig. 2 show little difference between Denmark and Switzerland, but in accordance with the low mortality from gastric cancer in England, the curve for this country is considerably lower. However, the exclusion of gastric cancer practically eliminates the differences among the three countries as shown in Fig. 3, and thus their differences in mortality from cancer in women must be ascribed to this type and to cancers of accessible sites, the curves of which are shown in Fig. 4.

Discussion.—Apart from differences caused by gastric cancer, cancers of inaccessible sites in women show no variation in mortality for the three countries. If diagnostic conditions were different in these countries they could be expected to cause deviations of just these curves. Therefore we may conclude that they are very much the same. Correspondingly, differences in diagnostic procedures are less important when we are dealing with accessible cancers, the mortality from which is more

likely to be influenced by differences in therapeutic results, or in cancer incidence.

The difference in female mortality from gastric cancer is responsible for the difference between Denmark and Switzerland, the two countries with highest mortality rates.

Males

Age distribution curves of mortality among men from cancer in the three countries examined are parallel but at different levels separated by statistically significant spaces as in paper I, Fig. 5 (1). The order of magnitude is the reverse of that for incidence among females, *i.e.* Switzerland, England and Denmark.

As demonstrated in Fig. 5 elimination of the values for gastric cancer from curves of total cancer mortality in males has the same effect as on the curves for females, as the difference between the intermediary English curve and the upper Swiss curve is almost eliminated.

But contrary to the results for inaccessible female cancers the exclusion of stomach cancer from the corresponding male group will, as seen in Figs. 6 and 7, result only in the transposition of the curves for England and Switzerland without other striking change.

The mortality curves for accessible cancers in males show the same order of magnitude for the three countries as the curves for total cancer.

Discussion.—As is the case with female cancers, the result of subtracting from the mortality figures for all cancers the figures for gastric cancer suggests that this form of cancer is mainly responsible for the difference between the two countries showing highest mortality from total cancer.

Cancers of both accessible and inaccessible sites contribute to the differences between the country of lowest mortality and the others.

SUMMARY

Continuing a series of studies on international cancer statistics, the authors have compared the official mortality rates for various age groups in Denmark, England and Switzerland from about 1938. The material was divided into cancers of accessible and inaccessible sites. Subtraction of the figures for gastric cancer from the total eliminated the differences of the mortality curves for all cancers for the two countries of highest mortality, namely Switzerland and England for men, and Denmark and England for women.

Furthermore, subtraction of the figures for gastric cancer from the mortality from all inaccessible cancers in women resulted in coincidence of the curves for all three countries. Consequently, it is suggested that diagnostic procedures are very much the same for women in the three countries, so that the deviations in mortality from accessible cancers are likely to be caused by differences either of therapeutic results or of cancer incidence.

The statistical differences found in the tables giving the values of u are designated as follows:

1. no sign: $u<1.96$; 5 per cent $< P$
2. ⊕ : $1.96< u<2.58$; 1 per cent $< P <5$ per cent
3. ⊕⊕ : $2.58< u<3.29$; 1 per thousand $< P <1$ per cent
4. ⊕⊕⊕ : $3.29< u$ $P<1$ per thousand.

where P is the probability of finding the observed or more widely differing values under the assumption that the true rates were the same in the groups compared.

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Table I: Cancer Mortality per 10,000 Living. Cancer of all Sites Except Stomach. Females

Age	Switzerland 1934–38	England and Wales 1937	Denmark 1935–39	England and Wales 1937	Denmark 1935–39
0–24	0.50		0.43	0.31	0.29
25–29		1.10		1.36	1.69
30–34	2.57		3.85		
35–39				4.29	5.34
40–44	9.38	11.3	12.1	8.39	9.09
45–49				14.4	15.5
50–54	22.8	25.6	25.7	22.3	21.1
55–59				29.2	30.8
60–64	38.6	44.2	42.8	39.3	37.9
65–69				50.3	49.3
70–74	67.8	77.9	73.9	71.6	66.8
75–79				85.2	84.4
80–84	88.1	105.3	100.5	96.5	97.2
85–				127.7	107.6

Note: Difficulties arose because the three countries under investigation had not applied similar age groupings. Consequently, it was necessary for us to work out separate groupings for the comparison of Danish and English statistics with those for Switzerland. It was especially complicated for the younger ages. Where the Swiss data yielded one group covering ages up to 29, those for England gave two groups, *i.e.*, 0–24 and 25–34. The way in which these difficulties have been overcome will be evident from the accompanying Tables.

TABLE IA: TEST OF SIGNIFICANCE. CANCER OF ALL SITES EXCEPT STOMACH. FEMALES

Values of u

Age	Switzerland and England	England and Denmark	Switzerland and Denmark
0–24		0.78	
25–29			1.42
30–34	− 1.48	− 2.76⊕⊕	
35–39			− 6.38⊕⊕⊕
40–44		− 3.41⊕⊕⊕	
45–49	− 5.92⊕⊕⊕	− 1.52	− 6.70⊕⊕⊕
50–54		− 1.91	
55–59	− 4.94⊕⊕⊕	1.50	− 4.28⊕⊕⊕
60–64		− 1.68	
65–69	− 6.43⊕⊕⊕	1.30	− 4.14⊕⊕⊕
70–74		0.60	
75–79	− 6.21⊕⊕⊕	2.28⊕	− 3.14⊕⊕⊕
80–84		0.29	
85–	− 4.63⊕⊕⊕	− 0.19	− 2.90⊕⊕
		2.86⊕⊕	

TABLE II: CANCER MORTALITY PER 10,000 LIVING. CANCER OF INACCESSIBLE SITES INCLUDING STOMACH. FEMALES

Age	Switzerland 1934–38	England and Wales 1937	Denmark 1935–39	England and Wales 1937	Denmark 1935–39
0–24	0.43		0.31	0.24	0.25
25–29		0.64		0.87	0.73
30–34	1.56		1.57		
35–39				2.05	2.26
40–44	5.30	4.96	5.98	3.63	4.51
45–49				6.38	5.89
50–54	16.5	13.7	15.7	11.1	12.1
55–59				16.4	19.9
60–64	38.2	30.1	37.8	25.0	30.3
65–69				36.6	47.4
70–74	79.9	61.0	84.8	55.4	73.6
75–79				70.1	101.5
80–84	95.0	75.5	115.4	74.2	117.5
85–				78.6	110.7

TABLE IIA: TEST OF SIGNIFICANCE. CANCER OF INACCESSIBLE SITES INCLUDING STOMACH. FEMALES

Values of u

Age	Switzerland and England	England and Denmark	Switzerland and Denmark
0–24		− 0.21	
25–29			3.20⊕⊕
30–34	1.98⊕	1.56	
35–39			− 0.06
40–44		− 0.98	
45–49	1.42	− 3.01⊕⊕	− 1.96⊕
50–54		1.26	
55–59	6.78⊕⊕⊕	− 1.68	1.36
60–64		− 4.72⊕⊕⊕	
65–69	10.77⊕⊕⊕	− 5.50⊕⊕⊕	0.42
70–74		− 7.96⊕⊕⊕	
75–79	12.12⊕⊕⊕	− 9.32⊕⊕⊕	− 2.44⊕
80–84		− 11.37⊕⊕⊕	
85–	5.66⊕⊕⊕	− 10.78⊕⊕⊕	− 4.14⊕⊕⊕
		− 5.26⊕⊕⊕	

TABLE III: CANCER MORTALITY PER 10,000 LIVING. CANCER OF INACCESSIBLE SITES. STOMACH EXCLUDED. FEMALES

Age	Switzerland 1934–38	England and Wales 1937	Denmark 1935–39	England and Wales 1937	Denmark 1935–39
0–24	0.42		0.29	0.23	0.24
25–29		0.52		0.68	0.59
30–34	1.23		1.20		
35–39				1.52	1.72
40–44	3.84	3.77	4.32	2.80	3.20
45–49				4.81	5.58
50–54	10.4	9.89	13.2	8.37	8.86
55–59				11.6	13.6
60–64	20.5	20.4	21.7	17.3	18.6
65–69				24.4	25.7
70–74	36.6	40.4	42.2	36.1	37.0
75–79				47.4	49.7
80–84	44.1	51.3	56.1	49.9	55.3
85–				54.5	57.8

TABLE IIIA: TEST OF SIGNIFICANCE. CANCER OF INACCESSIBLE SITES. STOMACH EXCLUDED. FEMALES

Values of u

Age	Switzerland and England	England and Denmark	Switzerland and Denmark
0–24		− 0.36	
25–29			3.36⊕⊕⊕
30–34	2.97⊕⊕	1.18	
35–39			0.25
40–44		− 1.18	
45–49	0.36	− 1.55	
50–54		− 2.18⊕	− 1.96⊕
55–59	1.42	− 1.06	
60–64		− 3.34⊕⊕⊕	− 5.82⊕⊕⊕
65–69	0.22	− 1.62	
70–74		− 1.22	− 1.60
75–79	− 3.29⊕⊕⊕	− 0.62	
80–84		− 1.04	− 3.93⊕⊕⊕
85–	− 2.74⊕⊕	− 1.70	
		− 0.66	− 3.90⊕⊕⊕

TABLE IV: CANCER MORTALITY PER 10,000 LIVING. CANCER OF ACCESSIBLE SITES. FEMALES

Age	Switzerland 1934–38	England and Wales 1937	Denmark 1935–39	England and Wales 1937	Denmark 1935–39
0–24	0.07		0.14	0.07	0.04
25–29		0.58		0.67	1.12
30–34	1.34		2.66		
35–39				2.79	3.62
40–44	5.54	7.50	7.78	5.59	5.89
45–49				9.54	9.92
50–54	12.4	15.3	14.6	13.9	12.3
55–59				16.8	17.3
60–64	18.2	23.8	21.1	22.1	19.2
65–69				25.9	23.6
70–74	31.3	37.5	31.8	35.6	29.8
75–79				40.5	34.9
80–84	43.9	54.5	44.4	46.6	41.9
85–				73.2	49.7

TABLE IVA: TEST OF SIGNIFICANCE. CANCER OF ACCESSIBLE SITES. FEMALES

Values of u

Age	Switzerland and England	England and Denmark	Switzerland and Denmark
0–24		1.56	
25–29			− 3.33⊕⊕⊕
30–34	− 5.46⊕⊕⊕	− 4.94⊕⊕⊕	
35–39		− 3.32⊕⊕⊕	− 8.38⊕⊕⊕
40–44		− 0.91	
45–49	− 7.47⊕⊕⊕	− 0.79	− 7.04⊕⊕⊕
50–54		2.82⊕⊕	
55–59	− 6.90⊕⊕⊕	− 0.66	− 4.44⊕⊕⊕
60–64		3.26⊕⊕	
65–69	− 9.30⊕⊕⊕	2.11⊕	− 4.10⊕⊕⊕
70–74		4.04⊕⊕⊕	
75–79	− 5.54⊕⊕⊕	2.88⊕⊕	− 0.36
80–84		1.68	
85–	− 3.98⊕⊕⊕	4.52⊕⊕⊕	− 0.18

TABLE V: CANCER MORTALITY PER 10,000 LIVING. CANCER OF ALL SITES EXCEPT STOMACH. MALES

Age	Switzerland 1934–38	England and Wales 1937	Denmark 1935–39	England and Wales 1937	Denmark 1935–39
0–24				0.40	0.30
25–29	0.57		0.36		
30–34		0.884		1.34	0.93
35–39	1.57		1.56		
40–44				2.42	1.93
45–49	6.37	6.59	4.84	4.74	3.67
50–54				8.54	6.18
55–59	24.9	20.4	14.55	15.8	11.1
60–64				27.3	18.5
65–69	55.8	56.7	36.6	47.0	30.1
70–74				69.6	45.1
75–79	101.2	104.5	77.3	95.7	67.8
80–84				121.0	91.9
85–	109.3	121.4	98.3	120.8	98.5
				122.2	97.8

TABLE VA: TEST OF SIGNIFICANCE. CANCER OF ALL SITES EXCEPT STOMACH. MALES

Values of u

Age	Switzerland and England	England and Denmark	Switzerland and Denmark
0–24		2.69⊕⊕	
25–29			4.81⊕⊕⊕
30–34	− 1.77	3.94⊕⊕⊕	
35–39		2.26⊕	0.09
40–44		3.38⊕⊕⊕	
45–49	− 0.74	5.22⊕⊕⊕	5.01⊕⊕⊕
50–54		7.28⊕⊕⊕	
55–59	8.13⊕⊕⊕	9.24⊕⊕⊕	16.13⊕⊕⊕
60–64		13.35⊕⊕⊕	
65–69	− 0.87	14.99⊕⊕⊕	16.04⊕⊕⊕
70–74		11.04⊕⊕⊕	
75–79	− 1.47	7.96⊕⊕⊕	9.93⊕⊕⊕
80–84		3.70⊕⊕⊕	
85–	− 2.26⊕	2.82⊕⊕	2.02⊕

TABLE VI: CANCER MORTALITY PER 10,000 LIVING. CANCER OF INACCESSIBLE SITES INCLUDING STOMACH. MALES

Age	Switzerland 1934–38	England and Wales 1937	Denmark 1935–39	England and Wales 1937	Denmark 1935–39
0–24				0.35	0.28
25–29	0.54		0.30		
30–34		0.84		1.25	0.67
35–39	1.75		1.42		
40–44				2.50	1.94
45–49	6.47	7.25	5.71	5.26	4.25
50–54				9.34	7.36
55–59	25.0	21.4	17.3	16.5	13.4
60–64				26.5	21.9
65–69	60.2	49.4	46.6	41.0	37.5
70–74				60.4	58.7
75–79	119.4	89.7	102.3	82.7	88.9
80–84				102.4	122.4
85–	120.3	102.8	120.3	103.5	122.2
				100.8	115.5

TABLE VIA: TEST OF SIGNIFICANCE. CANCER OF INACCESSIBLE SITES INCLUDING STOMACH. MALES

Values of u

Age	Switzerland and England	England and Denmark	Switzerland and Denmark
0–24		2.06⊕	
25–29			5.31⊕⊕⊕
30–34	− 0.44	5.94⊕⊕⊕	
35–39		2.54⊕	2.32⊕
40–44		2.92⊕⊕	
45–49	− 2.74⊕⊕	4.21⊕⊕⊕	2.36⊕
50–54		4.77⊕⊕⊕	
55–59	6.29⊕⊕⊕	5.20⊕⊕⊕	11.58⊕⊕⊕
60–64		2.79⊕⊕	
65–69	10.44⊕⊕⊕	1.04	10.76⊕⊕⊕
70–74		− 2.48⊕	
75–79	13.43⊕⊕⊕	− 5.50⊕⊕⊕	6.29⊕⊕⊕
80–84		− 3.48⊕⊕⊕	
85–	3.52⊕⊕⊕	− 1.71	0.0

TABLE VII: CANCER MORTALITY PER 10,000 LIVING. CANCER OF INACCESSIBLE SITES. STOMACH EXCLUDED. MALES

Age	Switzerland 1934–38	England and Wales 1937	Denmark 1935–39	England and Wales 1937	Denmark 1935–39
0–24				0.34	0.27
25–29	0.50		0.28		
30–34		0.68		0.99	0.50
35–39	1.22		1.01		
40–44				1.78	1.36
45–49	3.70	5.83	3.55	3.58	2.67
50–54				6.55	4.54
55–59	13.1	14.2	9.48	11.0	7.53
60–64				17.7	11.7
65–69	28.9	33.3	23.7	27.6	19.7
70–74				40.8	29.2
75–79	56.7	62.5	50.8	56.7	44.9
80–84				73.0	59.8
85–	60.3	76.1	60.2	74.5	60.9
				80.5	58.0

TABLE VIIa: TEST OF SIGNIFICANCE. CANCER OF INACCESSIBLE SITES. STOMACH EXCLUDED. MALES

Age	Values of u: Switzerland and England	England and Denmark	Switzerland and Denmark
0–24		1.87	
25–29			5.85⊕⊕⊕
30–34	0.14	5.68⊕⊕⊕	
35–39		2.24⊕	1.66
40–44	— 8.96⊕⊕⊕	3.28⊕⊕	0.61
45–49		5.16⊕⊕⊕	
50–54	— 2.66⊕⊕	6.47⊕⊕⊕	7.44⊕⊕⊕
55–59		8.46⊕⊕⊕	
60–64	— 5.48⊕⊕⊕	8.07⊕⊕⊕	5.80⊕⊕⊕
65–69		8.64⊕⊕⊕	
70–74	— 3.34⊕⊕⊕	5.96⊕⊕⊕	3.20⊕⊕⊕
75–79		4.58⊕⊕⊕	
80–84	— 3.90⊕⊕⊕	3.20⊕⊕	0.18
85–		3.30⊕⊕⊕	

TABLE VIII: CANCER MORTALITY PER 10,000 LIVING. CANCER OF ACCESSIBLE SITES. MALES

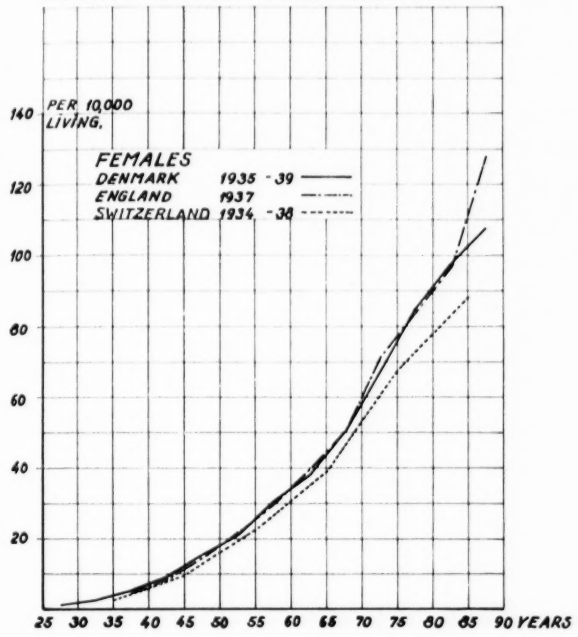
Age	Switzerland 1934–38	England and Wales 1937	Denmark 1935–39	England and Wales 1937	Denmark 1935–39
0–24	0.06	0.20	0.08	0.06	0.03
25–29				0.35	0.43
30–34	0.36		0.55		
35–39				0.64	0.57
40–44	2.67	1.57	1.30	1.16	1.00
45–49				2.00	1.63
50–54	11.8	7.07	5.07	4.80	3.53
55–59				9.52	6.83
60–64	26.9	23.4	12.8	19.3	13.3
65–69				28.7	15.9
70–74	44.5	42.2	26.5	39.0	22.8
75–79				47.9	32.1
80–84	49.0	45.2	38.2	46.4	37.4
85–				41.8	39.9

TABLE VIIIa: TEST OF SIGNIFICANCE. CANCER OF ACCESSIBLE SITES. MALES

Age	Values of u: Switzerland and England	England and Denmark	Switzerland and Denmark
0–24		2.19⊕	
25–29			— 1.78
30–34	— 3.91⊕⊕⊕	— 1.22	
35–39		0.64	— 2.46⊕
40–44	7.12⊕⊕⊕	0.96	7.40⊕⊕⊕
45–49		1.65	
50–54	12.36⊕⊕⊕	3.56⊕⊕⊕	16.22⊕⊕⊕
55–59		5.06⊕⊕⊕	
60–64	5.12⊕⊕⊕	7.36⊕⊕⊕	18.06⊕⊕⊕
65–69		11.56⊕⊕⊕	
70–74	1.60	10.36⊕⊕⊕	11.90⊕⊕⊕
75–79		7.04⊕⊕⊕	
80–84	1.16	2.71⊕⊕	3.27⊕⊕
85–		0.36	

1

CANCER OF ALL SITES EXCEPT STOMACH
MORTALITY RATES AT VARIOUS AGES



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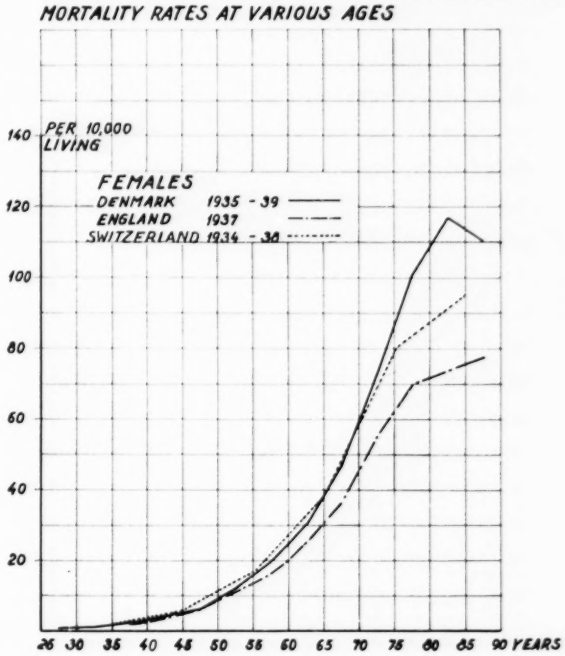
CANCER OF INACCESSIBLE SITES. INCLUDING STOMACH
MORTALITY RATES AT VARIOUS AGES



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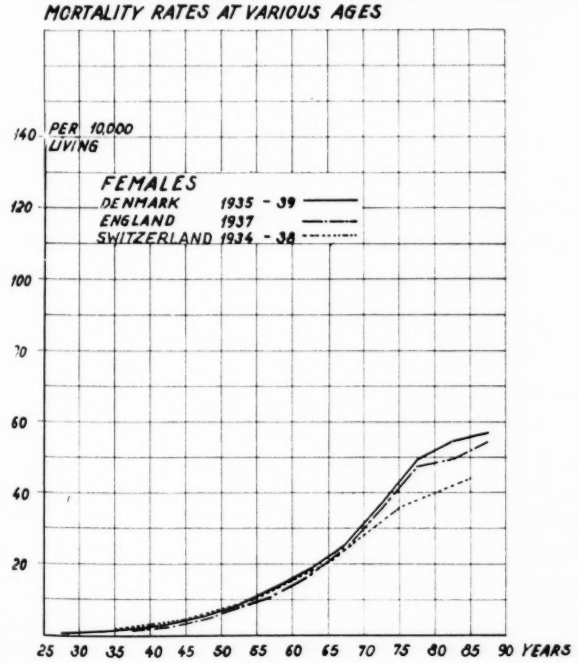
CANCER OF INACCESSIBLE SITES. STOMACH EXCLUDED
MORTALITY RATES AT VARIOUS AGES



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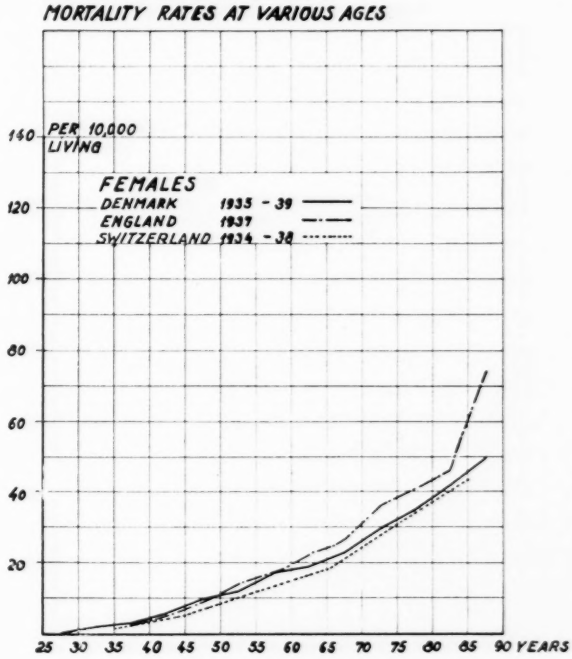
CANCER OF ACCESSIBLE SITES.
MORTALITY RATES AT VARIOUS AGES



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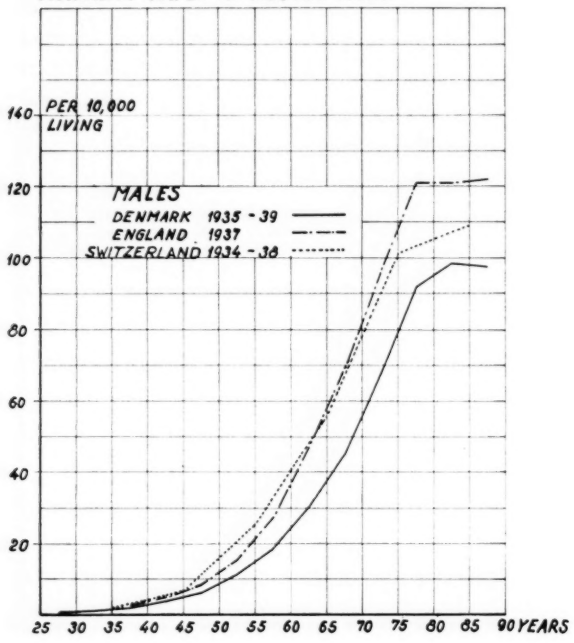
CANCER OF ALL SITES EXCEPT STOMACH
MORTALITY RATES AT VARIOUS AGES



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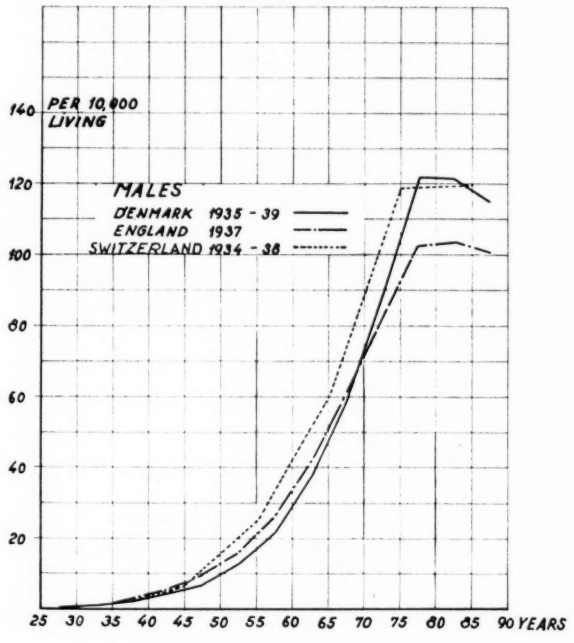
CANCER OF INACCESSIBLE SITES INCLUDING STOMACH
MORTALITY RATES AT VARIOUS AGES



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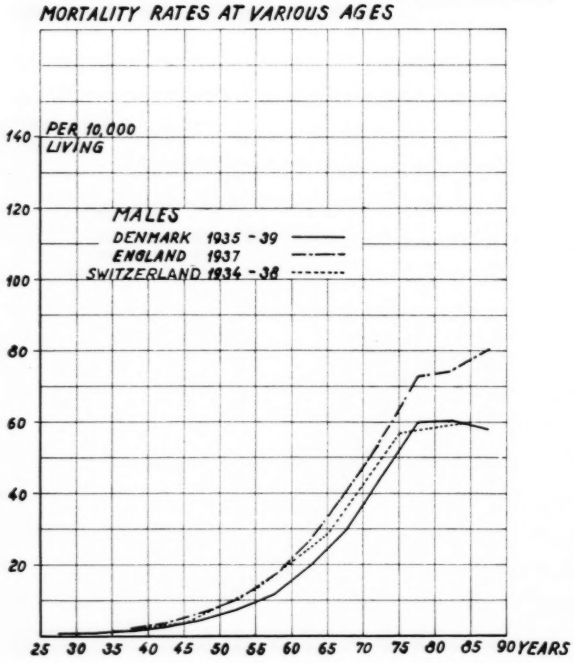
CANCER OF INACCESSIBLE SITES. STOMACH EXCLUDED
MORTALITY RATES AT VARIOUS AGES



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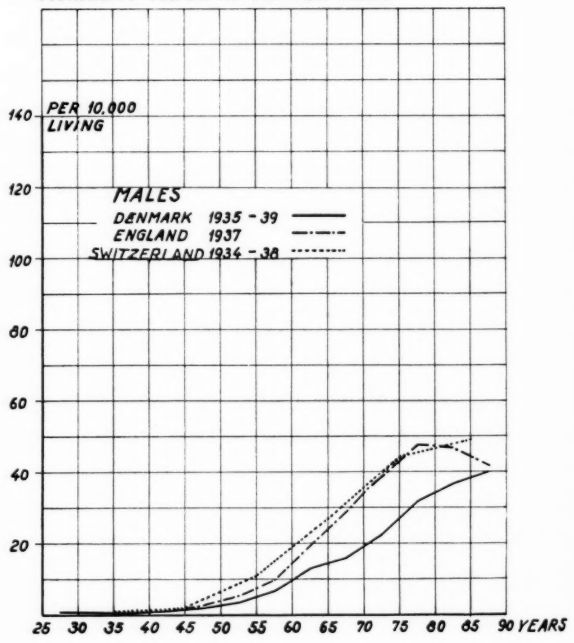
CANCER OF ACCESSIBLE SITES
MORTALITY RATES AT VARIOUS AGES



Note on the Heat Coagulation of Homogenates from Normal and Precancerous Livers*

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In connection with studies on riboflavin in various fractions of precancerous livers, the observation was made that homogenates of liver tissue from rats fed *m'*-methyl-*p*-dimethylaminoazobenzene failed to coagulate at 100° C., whereas similar homogenates from normal rats coagulated completely in 1 to 5 minutes. Accordingly a study was made of the stability towards heat of homogenates from livers of rats exposed to azo dyes for various lengths of time.

METHODS

Young adult rats of the Sprague-Dawley strain were fed a diet similar to that used in previous studies for the production of hepatic tumors with the azo dyes (10, 13). It had the following composition: casein, 12; salts, 4; corn oil, 5; and glucose monohydrate, 79. Appropriate amounts of the azo dyes, *p*-dimethylaminoazobenzene, aminoazobenzene, or *m'*-methyl-*p*-dimethylaminoazobenzene were dissolved in the oil to yield the concentrations desired (Tables I and II), and the feeding period was continued for intervals up to 16 weeks. The rats were then anesthetized with ether, the abdominal and thoracic cavities were exposed, and the liver perfused with 50 ml. of 0.85 per cent NaCl injected into the vena cava with the portal vein cut. The liver was then removed rapidly and homogenized for 3 minutes in a Waring blendor with 10 ml. of cold distilled water per gram of liver. Such homogenates were stable to centrifugation, to filtration through Whatman 42 filter paper, and to standing for 24 hours at room temperature.

For the coagulation studies 2 ml. aliquots were placed in 5 ml. test tubes and heated in a boiling water bath until visible coagulation occurred. Such coagula could be filtered or centrifuged off leaving a clear or relatively clear supernatant liquid. Homogenates were prepared from normal and precancerous livers and from tumors that had been induced with azo dyes or with methylcholanthrene and their coagulability determined either directly or after dilution with water or with homogenates from normal tissue. Homogenates from normal tissue were also diluted with water (Table I). In a more extensive study, various concentrations of the three azo dyes were fed to a larger group of rats and the animals killed at bi-weekly intervals up to 16 weeks and the coagulability of the livers determined as before.

RESULTS

Homogenates from the livers of rats not fed azo dyes coagulated completely within 1 to 3 minutes at the dilution used (10 cc. H_2O/gm. fresh tissue), whereas homogenates prepared from the livers of rats fed 0.064 per cent *m'*-methyl-*p*-dimethylaminoazobenzene for 6 weeks or more coagulated much more slowly or failed to coagulate after hours of heating (Table I). Homogenates prepared from hepatic tumors induced by the azo dye or from a rat sarcoma induced by the subcutaneous injection of methylcholanthrene showed a similar resistance to coagulation.

The stability of the homogenates to heat was found to depend upon the concentration of the homogenate and on the pH of the preparation. When a homogenate from normal liver coagulating in 1 minute was diluted with an equal part of water, coagulation was not complete until after 4 minutes of heating. A homogenate from a precancerous liver (0.064 per cent *m'*-methyl-*p*-dimethylaminoazobenzene fed for 2 months) that showed partial coagulation after 10 minutes of heating failed to coagulate in 30 minutes after it had been diluted with an equal part of water. On the other hand, an increase in acidity increased the coagulability of the homogenates. Homogenates that had been extremely resistant to coagulation, coagulated immediately under the influence of heat when the pH had been lowered by 0.8 unit. It is doubtful, however, that the difference between the normal and precancerous livers in the coagulability of their homogenates were due primarily to differences in acidity, since the pH of the homogenates from the two types of livers were essentially equal (pH range 7.0 to 7.2).

Mixtures of labile and resistant homogenates usually showed intermediate rates of coagulation

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when exposed to heat. A mixture of equal parts of coagulable and non-coagulable homogenates failed to coagulate, but in the presence of 2 parts of homogenate from normal liver and 1 from precancerous liver, coagulation was complete, although somewhat slower than that due to the normal liver alone (Table I). Homogenates from rat sarcoma agulation was rapid even after 16 weeks of exposure to the compound. On the other hand, in the presence of an equimolecular amount of the highly carcinogenic dye, *m'*-methyl-*p*-dimethylaminoazobenzene, some samples failed to coagulate after only 4 weeks of exposure to the compound. The feeding of *p*-dimethylaminoazobenzene, which

TABLE I: EFFECT OF DIETS CONTAINING *m'*-METHYL-*p*-DIMETHYLAMINOAZOBENZENE ON THE HEAT COAGULATION OF LIVER HOMOGENATES*

Diet	No. of rats	Degree of cirrhosis	Coagulation on heating
0.064% *m'*-Me-DAB†—6 weeks	10	moderate	none after 15 min.
Dye-free diet	10	none	complete—3 min.
A. 0.064% *m'*-Me-DAB—6 weeks	2	moderate	none—2 hrs.
B. Dye-free diet	1	none	complete—3 min.
2 parts A+1 part B			some—15 min.
3.3 parts A+1 part B			none—15 min.
0.5 " A+1 " B			complete—5 min.
1 " B+1 " H_2O			none—15 min.
A. 0.064% *m'*-Me-DAB—2 months	1	severe	some—10 min.
B. Dye-free diet	1	none	complete—1 min.
C. Rat sarcoma due to methylcholanthrene	1		none—1 hr.
1 part A+1 part H_2O			none—30 min.
1 part B+1 part H_2O			complete—4 min.
1 part B+1 part C			none—30 min.
0.064% *m'*-Me-DAB—10 weeks	1	moderate (non-tumorous part)	none—2 hrs.
Hepatic tumor from above			none—2 hrs.

* Homogenates = 10 cc. H_2O/gm. of fresh tissue.
† *m'*-Me-DAB = *m'*-methyl-*p*-dimethylaminoazobenzene.

TABLE II: RELATIVE EFFECT OF CERTAIN AZO DYES ON THE COAGULABILITY OF HOMOGENATES FROM RAT LIVER

Group No.	Diet and Dye Fed	2 weeks Liver	2 weeks Coagulation	4 weeks Liver	4 weeks Coagulation	6 weeks Liver	6 weeks Coagulation	8 weeks Liver	8 weeks Coagulation	12 weeks Liver	12 weeks Coagulation	16 weeks Liver	16 weeks Coagulation
1	No dye—*ad lib.*	Norm.	++++	Norm.	++++	Norm.	++++	Norm.	++++	Norm.	++++	Norm.	++++
2	No dye-restricted*	Norm.	++++	Fatty	+	Norm.	+++			Norm.	++++		
3	.096% *m'*-Me-DAB†	Mild cirrhosis‡	−										
4	.04% *m'*Me-DAB†			Norm.	++++	Norm.	++++			Mild cirrhosis	++		
5	.04% *m'*-Me-DAB† + 10 μg. B_{12}/gm.	Norm.	++++	Norm.	++++	Norm.	++++			Slight cirrhosis	++		
6	.064% *m'*-Me-DAB†	Norm.	++	Mild cirrhosis	−	Norm.	+++			Norm.	+++	Mild	−
					−	Cirrhotic	−	Cirrhotic	−	Mod. cirrhosis	−	cirrhosis	−
7	.06% DAB†	Norm.	++++	Norm.	++++	Near Normal	++	Norm.	++++	mild cirrhosis	−	Norm.	++++
8	.054% AAB†	Norm.	−	Norm.	++++	Norm.	++++	Norm.	++	Norm.	++++		

†DAB = *p*-dimethylaminoazobenzene
m'-Me-DAB = *m'*-methyl-*p*-dimethylaminoazobenzene.
AAB = Aminoazobenzene.
*Rats in group 2 received the same amount of food consumed by group 6.
‡Single survivor.

++++ Complete coagulation—1–3 min.
+++ Complete coagulation—3–6 min.
++ Complete coagulation—6–15 min.
+ Slight coagulation after 15 min.
− No coagulation—15 min.

likewise retarded the coagulation of homogenates from normal liver (Table I).

In the series in which azo dyes of various degrees of carcinogenic activity were fed (Table II) livers from the control animals not fed the dye coagulated rapidly on exposure to heat, as did livers from control animals fed only restricted amounts of food. An exception was noted in a single fatty liver, which did not coagulate as rapidly as the normal livers from rats on these diets. In the presence of the noncarcinogenic azo dye, aminoazobenzene, co- is definitely carcinogenic but only about half as active as the *m'*-methyl derivative, likewise resulted in reduced coagulability although a longer period of exposure to the dye was necessary (12 to 16 weeks) before the change became evident (Table II). The loss in coagulability seemed to parallel the gross physical condition of the liver: livers which on gross inspection showed a moderate number of cirrhotic nodules usually formed homogenates that failed to coagulate on exposure to heat; severely cirrhotic livers always failed to coagulate, whereas

homogenates from livers containing only a few cirrhotic nodules sometimes coagulated, although more slowly than normal.

DISCUSSION

A number of factors are probably responsible for the observed difference in coagulability of the homogenates of normal and precancerous liver. Others have reported that the addition of certain sugars (1, 3, 8) or fatty acids (2, 4-6, 9) may protect albumins against denaturation or coagulation with heat. Carter and Greenstein (7) observed that aqueous extracts of fresh liver from normal rats failed to coagulate when heated, but that they became heat-coagulable on standing at room temperature. The addition of thymus nucleic acid to such coagulable extracts prevented coagulation. Thymus nucleic acid also stabilized solutions of egg albumin which remained water clear for many hours at 98° C. when 1 mgm. of nucleate was present per 600 mgm. of protein. No experiments were reported on extracts from tumors or from precancerous livers.

That nucleic acid may have been one of the factors delaying coagulation in our present experiments is suggested by the observation that hepatic tumors due to azo dyes contain more nucleic acid than normal liver (12, 14). Lipids may also have been involved. Williams and associates (15) have reported that *p*-dimethylaminoazobenzene alters the distribution of lipids in the nucleus of the liver cell, with an increase in cholesterol ester and a significant decrease in phospholipid. But the most important factor is probably the concentration of coagulable protein present in the homogenate. The rate of coagulation of extracts from normal liver was shown to diminish as the concentration of total liver substance decreased. Morrione (11) has reported that the concentration of collagen is doubled in cirrhosis due to *p*-dimethylaminoazobenzene. The presence of the extra collagen would tend to delay coagulation of the entire extract by replacing, and therefore lowering the concentration of coagulable protein, and it might also delay coagulation by a more direct mechanism. Furthermore, the coagulable proteins in normal liver may have themselves undergone subtle changes under the influence of the azo dye. But whatever the mechanism involved, a simple measurement of coagulability may prove of value as a rapid index of the degree of liver damage that has taken place under any particular experimental regimen. This is suggested by the fact that delayed coagulation became evident about the same time that cirrhotic nodules appeared.

SUMMARY

1. Homogenates of livers from rats fed diets containing *m'*-methyl-*p*-dimethylaminoazobenzene for 6 weeks or longer failed to coagulate when heated in a boiling water bath. Normal liver homogenates coagulated completely after being heated for only a few minutes.

2. Resistance to coagulation paralleled the number of cirrhotic nodules. When 0.064 per cent *m'*-methyl-*p*-dimethylaminoazobenzene was fed, only 4 to 6 weeks were required before resistance to coagulation became marked, whereas 12 to 16 weeks were required before comparable resistance developed under the influence of 0.06 per cent *p*-dimethylaminoazobenzene. Rats fed the non-carcinogenic *p*-aminoazobenzene for 4 months were normal in appearance, and the homogenates prepared therefrom coagulated like those from normal livers.

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The Effect of Growth or Retrogression of a Transplantable Lymphosarcoma of the Rat on the Lymphoid Organs and the Adrenals of the Hosts

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The material used in this investigation was a rapidly growing, transplantable lymphosarcoma of the rat, which has been propagated in this laboratory since 1940 (2). The cells of this tumor inoculated intraperitoneally give rise to typical leukemia, but if inoculated into the subcutaneous tissue of the groin, a localized sarcoma develops that may metastasize to regional lymph nodes but rarely progress to generalized disease. At present, the tumor takes in from 60 to 70 per cent of the inoculated animals and, when progressive, grows rapidly to a very large size, causing death in about 20 days. In the routine autopsies on the inoculated rats, considerable variations were noted in the size of the lymphoid organs. An analysis of these observations follows.

OBSERVATIONS

The weights of the thymus, pooled cervical and axillary lymph nodes, spleen, and adrenals were taken on these organs from 159 rats made up of the following groups: (a) 53 rats with actively growing tumors, (b) 71 rats with tumors in various stages of retrogression, and (c) 35 normal rats of the same strain, age, and average weight, which had been kept under the same laboratory conditions as the inoculated animals.

The average weights of the organs from the three groups are given in Table I and the percentage variation from the normal is shown graphically in Fig. 1. It will be noted that the most pronounced difference is between the weight of the lymphoid organs of the rats with progressing tumors and those with retrogressing tumors. The average thymus weight of the group with progressing tumors, in the 31 of the 53 rats which showed detectable thymus tissue, was only 38 per cent of the average weight of the normal thymus. The average weight of this organ from animals with retrogressing tumors was increased by 49 per cent over the normal. The same general trend was observed in the lymph nodes, except that the average increase in weight of the nodes in rats with retrogressing tumors was more pronounced, being increased by 89 per cent over the normal weight. The spleen showed a comparable loss of weight in rats with growing tumors but in animals with retrogressing tumors the increase in the size of the spleen over the normal was not so pronounced as was that shown by the thymus and nodes. The organs from one group of animals are illustrated in Fig. 2.

In light of the previously reported indications (3, 4) that the adrenals and cortical hormones have an influence on the animal's resistance to this transplanted disease, the variations in average weights of these organs has an added interest. The glands from both the groups, one with growing tumors and the other with retrogressing tumors, showed a definite hypertrophy. In the former the increase in weight of the adrenals was 26.4 per cent over the normal, while in the latter the increase was 36.5 per cent. The average weights of adrenals from 16 rats in which the tumors had completely retrogressed were 55 per cent over the normal. Further analyses of the weights of adrenals from rats with retrogressing tumors show that those from animals killed 15 days after inoculation averaged 37.3 mgm. (22 rats) while those from animals killed from 25 to 29 days after averaged 49 mgm. (26 rats), an increase over the normal of 53 per cent.

DISCUSSION

The marked loss in weight of the principal lymphoid organs in animals with a rapidly growing lymphosarcoma may be the result of an uneven competition between the malignant and normal lymphoid cells for some essential nutritional factor. The definite hypertrophy of these organs, particularly the thymus and lymph nodes in rats with retrogressing lymphoid tumors, is not immediately explainable. In a previous study of the lymphoid organs in mice resistant to inoculated carcinoma, it was noted that there was a hyperactivity in the lymphoid tissues manifested by increase in mitoses, size of germinal centers, and numbers of cells in the circulation, but there was no consistent increase in weight of the lymphoid organs (1).

Excessive amounts of adrenal cortical hormone

may reduce the size of lymphoid organs to as great an extent as that which occurs in the animals with rapidly growing lymphosarcoma. Even though such animals have some hypertrophy of the adrenals it is doubtful whether this is sufficient to account for the extent of the change. An even greater hypertrophy of the gland in rats with retrogressing tumors is not sufficient to prevent the great increase in weight of thymus and nodes found in such animals. In fact, the extent of this increase in thymus and nodes is similar to that observed to follow the complete removal of the adrenals.

TABLE I

	No. of rats	Rats	Average weights Thymus	Nodes	Spleen	Adrenals
Normal	35	126 gm.	224.0 mgm.	177.2 mgm.	943.3 mgm.	31.9 mgm.
With progressive tumors	53	124	85.5*	96.9	422.6	40.4
With retrogressive tumors	71	123	335.1	334.9	1140.1	43.65

* No thymus in 41.5%.

SUMMARY

The weight of thymus, lymph nodes, and spleen from rats with actively growing transplanted lymphosarcoma are much reduced from the normal weights of these organs, the thymus by 62 per cent, nodes by 56 per cent, and the spleen by 55 per cent. Conversely, these organs are definitely increased in weight in rats bearing tumors in the process of retrogression. The lymph nodes show an increase of 89 per cent, the thymus 49 per cent and the spleen 21 per cent over the normal. The adrenals of rats with progressing tumors were found to average 25 per cent above the normal weights of these organs, while those from animals with retrogressing tumors had an increase of over 30 per cent. In the latter group the degree of hypertrophy was more marked when complete retrogression of the tumors had taken place (53 per cent).

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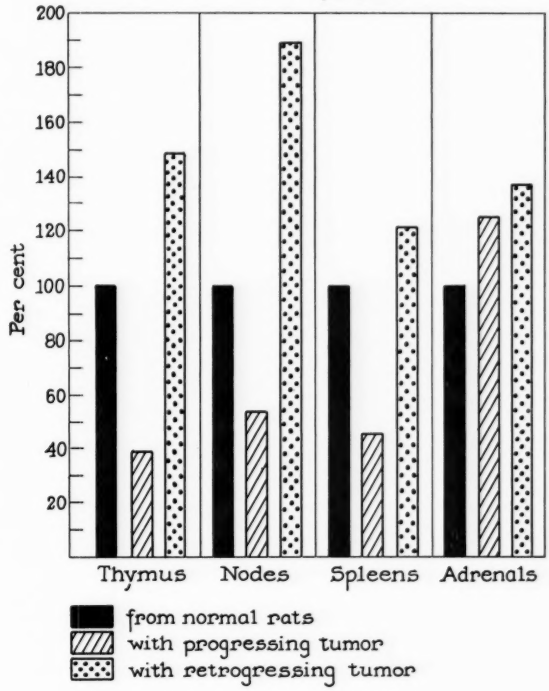
Figure 1
Percentage Variations from the Normal in Weights of Lymphoid Organs and Adrenals from Rats with Lymphosarcoma



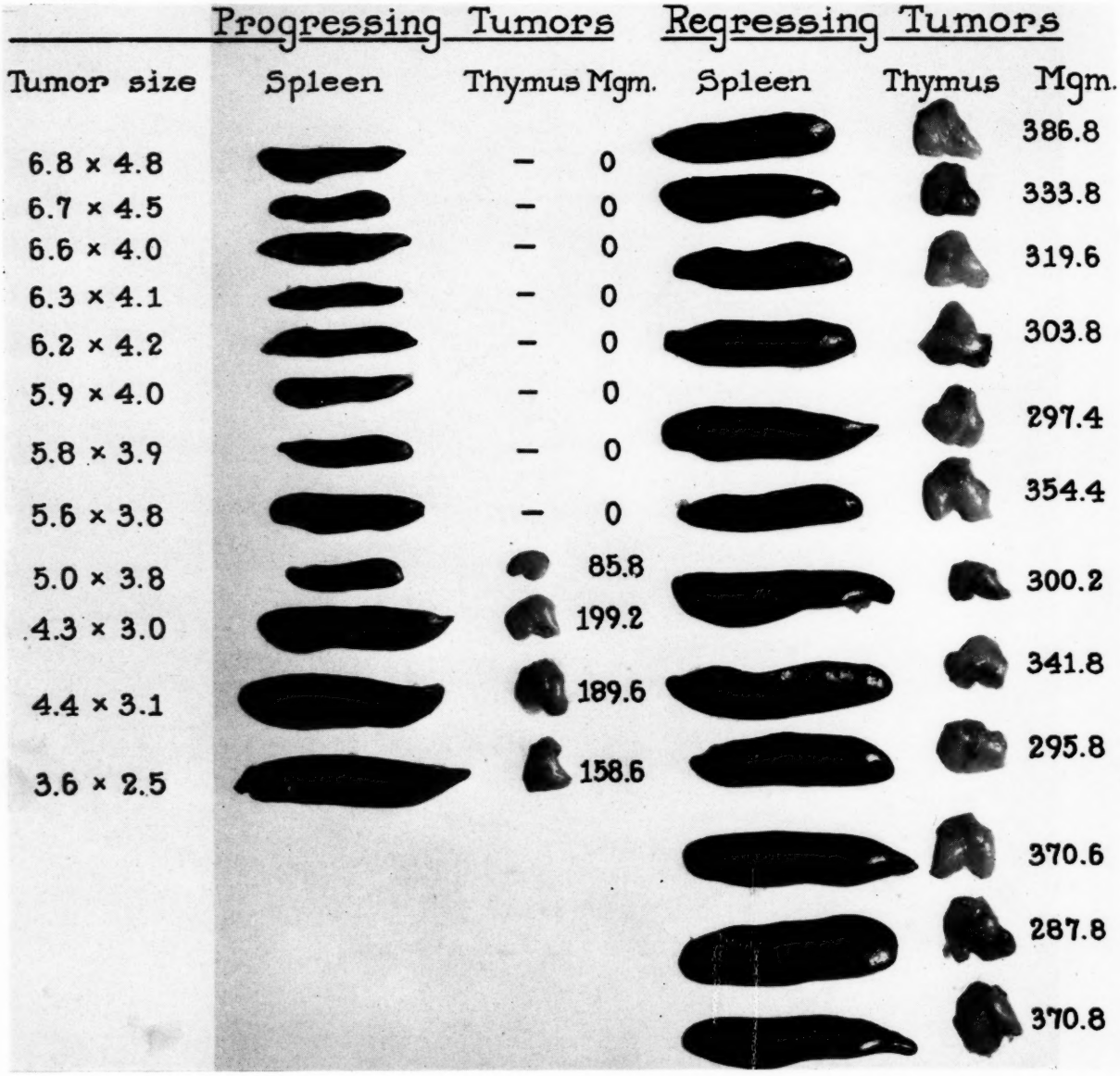

Progressing Tumors
Regressing Tumors
Tumor size
Spleen
Thymus Mgm.
Spleen
Thymus
Mgm.
6.8 × 4.8
– 0
386.8
6.7 × 4.5
– 0
333.8
6.6 × 4.0
– 0
319.6
6.3 × 4.1
– 0
6.2 × 4.2
– 0
303.8
5.9 × 4.0
– 0
5.8 × 3.9
– 0
297.4
5.6 × 3.8
– 0
354.4
5.0 × 3.8
85.8
300.2
4.3 × 3.0
199.2
4.4 × 3.1
189.6
341.8
3.6 × 2.5
158.6
295.8
370.6
287.8
370.8

FIG. 2

The Carcinogenicity of Certain Compounds Related to *p*-Dimethylaminoazobenzene*

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Many aminoazobenzene derivatives have been tested for carcinogenic activity in the rat (1-7). N,N-dimethyl-*p*-aminoazobenzene and N-methyl-*p*-aminoazobenzene have been found to be equally carcinogenic (4, 7). They produced cholangiomas and hepatomas in all animals tested in approximately the same period of time. N,N-dimethyl-3′-methyl-4-aminoazobenzene was more carcinogenic than the parent compound N,N-dimethyl-*p*-aminoazobenzene (4); but the N,N-dimethyl-2′-methyl-4-aminoazobenzene and N,N-dimethyl-4′-methyl-4-aminoazobenzene were very much less active. N,N-diethyl-*p*-aminoazobenzene and all other higher alkyl homologues of N,N-dimethyl-*p*-aminoazobenzene tested failed to produce cirrhosis or neoplastic changes in the liver of the rat when fed in equimolecular amounts (7).

The investigation has been extended to several compounds of this series which have not been previously tested.

EXPERIMENTAL

A 3 per cent solution of N,N-dimethyl-*p*-aminoazobenzene or molar equivalent amounts of the other compounds were prepared with cottonseed oil, and 20 cc. were evenly mixed with 1,000 gm. of coarsely ground, unpolished rice; *i.e.*, 0.6 gm. of the compound per kgm. The rats were permitted to eat as much of the mixture as they desired; all received a small amount of fresh carrots daily and unlimited quantities of water were permitted. Feeding was continued until the animals either died or were sacrificed at the end of the experimental period of 250 days. The rats used in this study were Sherman strain males weighing about 150 gm.

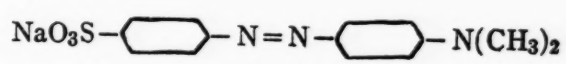
The results obtained are summarized in Tables I and II. In all cases the gross diagnosis was confirmed by microscopic examination.

Although the number of days of feedings was the same, individual rats consumed different amounts of azo compounds (6). Therefore, we have calculated the approximate amounts of azo compounds consumed daily by each animal from the average amount ingested by a group of rats at different times during the experimental period. These figures are given in the fifth column of Table II.

As may be seen from the data in Tables I and II, N-methyl-3′-methyl-4-aminoazobenzene was at least as carcinogenic as the N-methyl or N,N-dimethyl-*p*-aminoazobenzene. The animals fed N-methyl-3′-methyl compound developed typical liver cancer in all cases during experimental periods of 141 to 225 days. However, the N-methyl-2′-methyl-4-aminoazobenzene and the N-methyl-4′-methyl-4-aminoazobenzene were very much less carcinogenic, incidence of liver cancer being 27 and 20 per cent, respectively, during the experimental period of 100 to 250 days.

N,N-diethyl-*p*-aminoazobenzene failed to produce cirrhosis or liver tumors, but N-methyl-N-ethyl-*p*-aminoazobenzene was definitely carcinogenic (67 per cent liver cancer by 250 days), an indication of the importance of the methyl radical for carcinogenesis. N,N-diethanol-*p*-aminoazobenzene was similarly tested for its carcinogenicity. None of the 10 rats fed the compounds showed cirrhosis or neoplastic changes in the liver during 313 days.

It is of interest that addition of a hydroxy group in the para prime position of *p*-dimethylaminoazobenzene resulted in a compound possessing no carcinogenicity. The livers of rats fed N,N-dimethyl-4′-hydroxy-4-aminoazobenzene had smooth surfaces and histological examination showed no evidence of tumors, bile duct changes, or abnormal regeneration of the ducts and liver cells, or any abnormal nuclear alteration. Introduction of a water-soluble group in the 4′ position may change the character of a carcinogenic compound, *p*-dimethylaminoazobenzene. Thus, methyl orange,



which is water-soluble, is not carcinogenic (2).

Unfortunately many of the animals fed N,N-dimethyl-4′-hydroxy-4-aminoazobenzene died within 150 days without noteworthy liver change or other visceral changes except congestion of the lungs. Therefore, the experiment was repeated using heavier rats, about 200 gm., but the toxic effect

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of the compound on the animal did not diminish. Of the 10 rats, 8 died between 95 and 150 days after the beginning of the experiment and the remaining 2 rats were sacrificed on the 212th day. Microscopic examination of the liver revealed neither cirrhosis nor tumor formation.

The failure of N,N-dimethyl-4′-hydroxy-4-aminoazobenzene to produce liver tumors may lie in the fact that only 4 animals lived over 150 days. A small probability of liver tumor production may have been missed because of the small number of survivors. Of course the compound may be noncarcinogenic.

Nutrition of young adult rats during ingestion of the N,N-dimethyl-*p*-aminoazobenzene, N-methyl-*p*-aminoazobenzene, N-methyl-2′-methyl-4-aminoazobenzene, N-methyl-3′-methyl-4-aminoazobenzene and N-methyl-4′-methyl-4-aminoazobenzene in addition to the rice diet with supplement of fresh carrots, may be briefly summarized as follows: The majority of the rats fed N,N-dimethyl-*p*-aminoazobenzene, N-methyl-*p*-aminoazobenzene and N-methyl-3′-methyl-4-aminoazobenzene lost body weight from the beginning of the experiment. Some gained weight slightly during the first 2 weeks, and maintained their weight for about 2 months. At this time, the general appearance of the animals was good, but thereafter, when malig-

TABLE I: INCIDENCE OF HEPATIC TUMORS IN RATS FED VARIOUS AZO COMPOUNDS

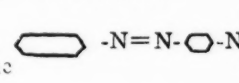
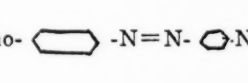
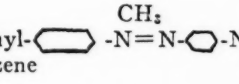
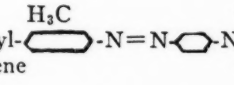
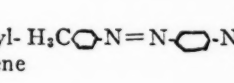
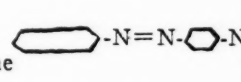
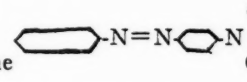
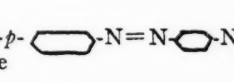
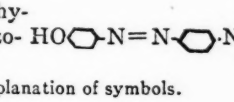
Compound fed	No. of animals	Liver findings at autopsy*								
N,N-Dimethyl-*p*-aminoazobenzene	15	+ 95, ++ 168,	± 101, +++ 168,	+ 115, +++ 168,	+ 137, + 192,	+ 150, ++ 200,	++ 150, +++ 250 days	++ 150,	+++ 153,	++ 166,
N-Methyl-*p*-aminoazobenzene	14	+ 132, + 190,	+++ 147, +++ 200,	+++ 155, ± 237,	++ 157, + 250,	+ 171, +++ 250 days	++ 177,	+++ 181,	++ 182,	+ 183,
N-Methyl-2′-methyl-4-aminoazobenzene	15	− 98, +++ 230,	− 107, − 234,	− 124, ± 237,	− 127, − 247,	+ 138, − 247,	+ 184, − 250 days	− 204,	− 211,	+ 230,
N-Methyl-3′-methyl-4-aminoazobenzene	15	++ 141, ++ 171,	+++ 141, + 172,	+ 145, ++ 173,	+ 147, ++ 174,	+++ 151, + 175,	++ 156, +++ 225 days	++ 156,	+ 161,	+ 167,
N-Methyl-4′-methyl-4-aminoazobenzene	15	± 100, − 250,	+ 189, − 250,	− 199, − 250,	± 202, − 250,	− 234, ± 250,	+ 237, + 250 days	− 247,	± 247,	− 250,
N,N-Diethyl-*p*-aminoazobenzene	20	− 123, − 289, − 313,	− 162, − 313, − 313 days	− 166, − 313,	− 171, − 313,	− 228, − 313,	− 235, − 313,	− 238, − 313,	− 240, − 313,	± 240, − 313,
N,N-Diethanol-*p*-aminoazobenzene	10	− 162, − 313 days	− 165,	− 175,	− 228,	− 289,	− 313,	− 313,	− 313,	− 313,
N-Methyl-N-ethyl-*p*-aminoazobenzene	15	− 110, − 250,	+ 133, − 250,	++ 151, − 250,	+ 182, − 250,	− 199, − 250,	+ 200, − 250 days	+ 202,	++ 232,	++ 236,
N,N-Dimethyl-4′-hydroxy-4-aminoazobenzene	16	− 87, − 116,	− 90, − 120,	− 98, − 121,	− 98, − 121,	− 106, − 121,	− 111, − 250,	− 112, − 250 days	− 115,	− 116,

* − indicates smooth, practically normal liver; ± indicates nodular cirrhosis with adenomatous hyperplasia; + indicates distinct areas of cholangioma or hepatoma or both; ++ indicates extensive liver cancer without metastasis; +++ indicates extensive liver cancer with metastasis.

nant changes began to appear in the liver, the condition of the animals was poor. On the other hand, animals on N-methyl-2′-methyl-4-aminoazobenzene and N-methyl-4′-methyl-4-aminoazobenzene gained weight or maintained their body weight during the first 2 months. About 70 per cent of the animals presented a healthy appearance throughout the experimental period of 250 days.

Nutrition of young adult rats during ingestion of N,N-diethyl-*p*-aminoazobenzene and N,N-diethanol-*p*-aminoazobenzene was good. Practically all animals gained weight and presented a healthy appearance throughout the experimental period of 313 days. Rats fed N-methyl-N-ethyl-*p*-aminoazobenzene-rice diet maintained their body weight during the first 2 months, and thereafter their physical appearance was poor except in about 30 per cent of the animals, which did not develop liver tumors.

TABLE II. INCIDENCE OF HEPATIC TUMORS IN RATS FED VARIOUS AZO COMPOUNDS

Chemical compound	Structural formula	No. of animals	Per cent in diet	Amount ingested daily, mgm.	No. of days fed	Liver findings*					Incidence of liver cancer, per cent
						−	±	+	++	+++	
N,N-Dimethyl-*p*-aminoazobenzene		15	0.06	4.8	95–250	0	1	5	5	4	93
N-Methyl-*p*-aminoazobenzene		14	0.056	4.8	132–250	0	1	5	3	5	93
N-Methyl-2′-methyl-4-aminoazobenzene		15	0.06	6.0	98–250	10	1	3	0	1	27
N-Methyl-3′-methyl-4-aminoazobenzene		15	0.06	4.5	141–225	0	0	6	6	3	100
N-Methyl-4′-methyl-4-aminoazobenzene		15	0.06	6.0	100–250	8	4	3	0	0	20
N-N,-Diethyl-*p*-aminoazobenzene		20	0.067	7.4	123–313	19	1	0	0	0	0
N,N-Diethanol-*p*-aminoazobenzene		10	0.076	8.4	162–313	10	0	0	0	0	0
N-Methyl-N-ethyl-*p*-aminoazobenzene		15	0.064	4.8	110–250	5	0	6	4	0	67
N,N-Dimethyl-4′-hydroxy-4-aminoazobenzene		16	0.064	4.0	87–250	16	0	0	0	0	0

* See Table I for explanation of symbols.

liver cancer, but daily ingestion of about 4.8 mgm. of N-methyl-N-ethyl-*p*-aminoazobenzene produced liver cancer, an indication of the importance of the methyl radical for carcinogenesis.

4. An hydroxy group in the para prime position of *p*-dimethylaminoazobenzene resulted in a compound possessing no carcinogenicity under these conditions.

SUMMARY

1. Nearly all of the rats which were fed about 4.8 mgm. of N,N-dimethyl-*p*-aminoazobenzene or N-methyl-*p*-aminoazobenzene daily for periods of 100 to 250 days developed hepatic cancer.

2. Although N-methyl-3′-methyl-4-aminoazobenzene is at least as carcinogenic as the N-methyl or N,N-dimethyl compound, is isomers: N-methyl-2′-methyl-4-aminoazobenzene and N-methyl-4′-methyl-4-aminoazobenzene, are much less carcinogenic.

3. Daily ingestion of about 7.4 mgm. of N,N-diethyl-*p*-aminoazobenzene or about 8.4 mgm. of N,N-diethanol-*p*-aminoazobenzene failed to produce

ACKNOWLEDGMENT

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